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National Toxicology Program
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NTP MONOGRAPH ON HEALTH EFFECTS OF LOW-LEVEL LEAD

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Office of Health Assessment and Translation
Division of the National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

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PEER REVIEW OF THE DRAFT NTP MONOGRAPH ON HEALTH EFFECTS OF LOW-LEVEL LEAD

Peer review of the Draft NTP Monograph on Health Effects of Low-level Lead was conducted by an *ad hoc* expert panel at a public meeting held November 17-18, 2011, at the National Institute of Environmental Health Sciences, Research Triangle Park, NC (see *http://ntp.niehs.nih.gov/go/37090* for materials, minutes, and panel recommendations from the peer review meeting). The selection of panel members and conduct of the peer review were performed in accordance with the Federal Advisory Committee Act and Federal policies and regulations. The panel members served as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members had two major responsibilities in reviewing the draft NTP Monograph: (1) to determine whether the scientific information cited in the draft monograph is technically correct, clearly stated, and objectively presented and (2) to determine whether the scientific evidence presented in the draft monograph supports the NTP's conclusions regarding health effects of low-level lead (Pb).

The panel agreed with the draft NTP overall conclusions on cardiovascular, renal, and immune health effects associated with blood Pb levels <10 µg/dL. The panel recommended changing the draft summary conclusion for neurological effects in children and for reproductive effects in adult women from *sufficient* evidence of an association at blood Pb levels <10 ug/dL to *sufficient* evidence of an association at blood Pb levels <5 ug/dL. Comments from the peer reviewers and written public comments received on the draft monograph were considered during finalization of the document. The NTP concurred with the expert panel on all of its recommendations on the conclusions regarding health effects of Pb in this final document.

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ABBREVIATIONS

ABLES	Adult Blood Lead Epidemiology and Surveillance
ACCLPP	Advisory Committee on Childhood Lead Poisoning Prevention
ADHD	attention deficit hyperactivity disorder
ALAD	δ-aminolevulinic acid dehydratase
ALS	amyotrophic lateral sclerosis
AQCD	Air Quality Criteria Document
ATSDR	Agency for Toxic Substances and Disease Registry
BAEP	brainstem auditory evoked potential
BMI	body mass index
BP	blood pressure
CBCL	Child Behavior Checklist
BTQ	Boston Teacher Questionnaire
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CKD	chronic kidney disease
DBP	diastolic blood pressure
DSM	*Diagnostic and Statistical Manual of Mental Disorders*
DSM-IV	*Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (1994)
DSM-IV-TR	*Diagnostic and Statistical Manual of Mental Disorders*, 4th edition, text revision (2000)
DTH	delayed-type hypersensitivity
E_2	estradiol-17β
EBE	early biological effect marker
ECG	electrocardiographic
EDTA	ethylenediaminetetraacetic acid
eGFR	estimated glomerular filtration rate
EPA	Environmental Protection Agency, U.S.
ERG	electroretinographic
ETS	environmental tobacco smoke
FR	fecundability ratio
FSH	follicle-stimulating hormone
FSIQ	full-scale IQ
GCI	General Cognitive Index from the McCarthy Scales of Children's Abilities
GFR	glomerular filtration rate
HFE	hemochromatosis
HR	hazard ratio
HRV	heart rate variability
IgA	immunoglobulin A
IgE	immunoglobulin E
IGF-1	insulin-like growth factor 1
IgG	Immunoglobulin G
IgM	immunoglobulin M

IQ	intelligence quotient
IVF	in vitro fertilization
KTEA	Kaufman Test of Educational Achievement
LH	leutinizing hormone
MDI	Mental Developmental Index from the Bayley Scales of Infant Development
MMSE	Mini-Mental State Examination
NAG	N-acetyl-β-D-glucosaminidase
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NO	nitric oxide
NTP	National Toxicology Program
Pb	lead
O_2^-	superoxide
OR	odds ratio
PBPK	physiologically based pharmacokinetic
PFC	plaque-forming cell
PIQ	performance IQ
PRL	prolactin
RBP	retinol-binding protein
RR	relative risk
SBP	systolic blood pressure
SD	standard deviation
SES	socioeconomic status
SPT	skin prick test
T	testosterone
T_3	triiodothryonine
T_4	thyroxin
Th-1	type 1 helper T-cell
Th-2	type 2 helper T-cell
TNF-α	tumor necrosis factor-α
TSH	thyroid-stimulating hormone
VEP	visual evoked potential
VDR	vitamin D receptor
VIQ	verbal IQ
WHILA	Swedish Women's Health in the Lund Area
WISC	Wechsler Intelligence Scale for Children
WISC-III	Wechsler Intelligence Scale for Children, 3rd edition (1991)
WISC-R	Wechsler Intelligence Scale for Children, revised edition (1974)
WISC-IV	Wechsler Intelligence Scale for Children, 4th edition (2003)
WPPSI-R	Wechsler Preschool and Primary Scale of Intelligence
WRAT-R	Wide Range Achievement Test-Revised
ZPP	zinc protoporphyrin

ABSTRACT

Although reductions in lead (Pb) exposure for the U.S. population have resulted in lower blood Pb levels over time, epidemiological studies continue to provide evidence of health effects at lower and lower blood Pb levels. Low-level Pb was selected for evaluation by the National Toxicology Program (NTP) because of (1) the availability of a large number of epidemiological studies of Pb, (2) a nomination by the National Institute for Occupational Safety and Health for an assessment of Pb at lower levels of exposure, and (3) public concern for effects of Pb in children and adults. This evaluation summarizes the evidence in humans and presents conclusions on health effects in children and adults associated with low-level Pb exposure as indicated by less than 10 micrograms of Pb per deciliter of blood (<10 μg/dL). The assessment focuses on epidemiological evidence at blood Pb levels <10 μg/dL and <5 μg/dL because health effects at higher blood Pb levels are well established. The NTP evaluation was conducted through the Office of Health Assessment and Translation (OHAT, formerly the Center for the Evaluation of Risks to Human Reproduction) and completed in April of 2012.

The results of this evaluation are published in the NTP Monograph on Health Effects of Low-Level Lead. The document and appendices are available at *http://ntp.niehs.nih.gov/go/evals*. This document provides background on Pb exposure and includes a review of the primary epidemiological literature for evidence that low-level Pb is associated with neurological, immunological, cardiovascular, renal, and/or reproductive and developmental effects. The NTP Monograph presents specific conclusions for each health effect area. Overall, the NTP concludes that there is *sufficient* evidence that blood Pb levels <10 μg/dL and <5 μg/dL are associated with adverse health effects in children and adults.

This conclusion was based on a review of the primary epidemiological literature, scientific input from technical advisors that reviewed pre-public release drafts of each chapter summarizing the evidence for specific health effects associated with low-level Pb, public comments received during the course of the evaluation, and comments from an expert panel of *ad hoc* reviewers during a public meeting to review the draft NTP Monograph on health effects of Low-level Lead on November 17-18, 2011 (*http://ntp.niehs.nih.gov/go/37090*).

1.0 EXECUTIVE SUMMARY

1.1 Introduction

Lead (Pb) exposure remains a significant health concern despite policies and practices that have resulted in continued progress in reducing exposure and lowering blood Pb levels in the U.S. population. Pb is one of the most extensively studied environmental toxicants, with more than 28,900 publications on health effects and exposure in the peer-reviewed literature.[1] While the toxicity associated with exposure to high levels of Pb was recognized by the ancient Greeks and Romans, the adverse health effects associated with low-level Pb exposure became widely recognized only in the second half of the 20th century. Over the past 40 years, epidemiological studies, particularly in children, continue to provide evidence of health effects at lower and lower blood Pb levels. In response, the Centers for Disease Control and Prevention (CDC) has repeatedly lowered the concentration of Pb in blood that is considered "elevated" in children (from 30 µg/dL to 25 µg/dL in 1985 and to the current level of 10 µg/dL in 1991).

The purpose of this evaluation is to summarize the evidence in humans and to reach conclusions about whether health effects are associated with low-level Pb exposure as indicated by less than 10 micrograms of Pb per deciliter of blood (<10 µg/dL), with specific focus on the life stage (childhood, adulthood) associated with these health effects. This evaluation focuses on epidemiological evidence at blood Pb levels <10 µg/dL because health effects at higher blood Pb levels are well established such that the definition of an elevated blood Pb level is ≥10 µg/dL for both children and adults (ABLES 2009, CDC 2010a). Pb was nominated by the National Institute for Occupational Safety and Health for a National Toxicology Program (NTP) evaluation to assess the reproductive and developmental effects of Pb (see http://ntp.niehs.nih.gov/mtg?date=20100510&meeting=BSC). The scope of the evaluation has been expanded from the original nomination to include an evaluation of health effects other than reproduction and development (e.g., cardiovascular effects in adults) in order to maximize the utility of the evaluation.

1.2 Methods

The key questions and general approach for developing the conclusions on the health effects of low-level Pb are outlined below. **Section 2.0** of this document contains additional details on the authoritative sources considered, the literature search strategy, and the peer-review process.

1.2.1 Key Questions

What is the evidence that adverse health effects are associated with blood Pb <10 µg/dL?

- What reproductive, developmental, neurological, immune, cardiovascular, and renal health effects are associated with blood Pb levels <10 µg/dL?
- What is the blood Pb level associated with a given health effect (i.e., <10 µg/dL or <5 µg/dL)?

[1] Based on an April 2012 PubMed search for keyword (MeSH) "lead" or "lead poisoning."

- At which life stages (childhood or adulthood) is the effect identified?
- Are there data to evaluate the association between bone Pb and the health effect, and how does the association to this biomarker of Pb exposure compare to the association with blood Pb?

1.2.2 Approach to Develop Health Effects Conclusions

Conclusions in the NTP evaluation of Pb-related health effects in humans associated with low-level Pb were derived by evaluating the data from epidemiological studies with a focus on blood Pb levels <10 µg/dL. The evaluation includes a review of the primary epidemiological literature for evidence that low-level Pb is associated with neurological, immunological, cardiovascular, renal, and/or reproductive and developmental effects. These health effect areas were selected because there is a relatively large database of human studies in each area. The NTP considered four possible conclusions for specific health effects within each area:

> **Sufficient Evidence of an Association**: an association is observed between the exposure and health outcome in studies in which chance, bias, and confounding could be ruled out with reasonable confidence.
>
> **Limited Evidence of an Association**: an association is observed between the exposure and health outcome in studies in which chance, bias, and confounding could not be ruled out with reasonable confidence.
>
> **Inadequate Evidence of an Association**: the available studies are insufficient in quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an association between exposure and health outcome, or no data in humans are available.
>
> **Evidence of No Association:** several adequate studies covering the full range of levels of exposure that humans are known to encounter (in this case limited to blood Pb levels <10 µg/dL) are mutually consistent in not showing an association between exposure to the agent and any studied endpoint.

The discussion of each health effect begins with a statement of the NTP's conclusion regarding whether the specific effect is associated with a blood Pb level <10 µg/dL or <5 µg/dL and the age group (childhood or adulthood) in which it is or is not identified, as well as the timing of exposure associated with the effect (prenatal, childhood, concurrent) if available. Then key data and principal studies considered in developing the NTP's conclusions are discussed in detail. General strengths and limitations of study designs were considered when developing conclusions, with prospective studies providing stronger evidence than cross-sectional or case-control studies. Each section concludes with a summary discussing each health effect, describing experimental animal data that relate to the human data, and stating the basis for the NTP conclusions.

For the purposes of this evaluation, "children" refers to individuals <18 years of age unless otherwise specified. In addition to the blood Pb level of <10 µg/dL, a lower effect level of

<5 µg/dL was also selected because it is commonly used in epidemiological studies to categorize health effects data by exposure levels; therefore, data are often available to evaluate health effects for groups above and below this value as well.

1.2.3 Appendices of Studies Considered

The information to support the NTP's conclusions for individual health effects is presented in each chapter. In addition, human studies of groups with low-level Pb exposure that were considered in developing the conclusions are also abstracted for further reference and included in separate appendices for neurological effects, immune effects, cardiovascular effects, renal effects, and reproductive and developmental effects.

1.2.4 Authoritative Sources and Peer Review

In this evaluation, the NTP made extensive use of recent government assessments of the health effects of Pb, especially the U.S. Environmental Protection Agency (EPA) 2006 Air Quality Criteria Document (AQCD) for Lead (U.S. EPA 2006 and a draft updated version, 2012), which has undergone extensive external public peer review. In addition to the EPA's 2006 AQCD for Lead, sources include the Agency for Toxic Substances and Disease Registry's (ATSDR) 2007 Toxicological Profile for Lead (ATSDR 2007) and the CDC's Advisory Committee on Childhood Lead Poisoning Prevention reports, such as the 2010 Guidelines for the Identification and Management of Lead Exposure in Pregnant and Lactating Women (CDC 2010b).

The NTP used independent subject matter experts as technical advisers to provide scientific input and to review pre-public release drafts of each chapter summarizing the evidence that health effects are associated with low-level Pb, the appendices, and **Section 3.0** that provides background on Pb exposure (see **Contributors** for a list of technical advisers). Peer review of the draft document was conducted by an expert panel of *ad hoc* reviewers at a public meeting held November 17-18, 2011, in Research Triangle Park, NC (see **Peer-Review of the Draft NTP Monograph on Health Effects of Low-level Lead** for details). Comments from peer reviewers and written public comments received on the draft monograph were considered during finalization of the document. The NTP concurred with the expert panel on all of the conclusions regarding health effects of Pb in this final document.

1.3 What Does It Mean to Refer to Blood Pb Levels <10 µg/dL?

The overwhelming majority of human epidemiological studies with Pb exposure data measured Pb in whole blood, and this measure of exposure serves as the basis for the evaluation of Pb levels <10 µg/dL. An individual's blood Pb level reflects an equilibrium between current environmental Pb exposure and the preexisting amount of Pb in the body, stored primarily in bone (Factor-Litvak *et al.* 1999, Brown *et al.* 2000, Chuang *et al.* 2001). In adults, bone and teeth store 90-95% of the total body burden of Pb, while in young children, bone Pb represents a smaller fraction (down to 70%) (Barry 1981, for review, see Barbosa *et al.* 2005, Hu *et al.* 2007). The body eliminates half of the Pb in circulating blood (half-life) in approximately one month, while bone is a more stable repository for Pb and, therefore, bone Pb levels reflect cumulative exposure to Pb integrated over years or even decades (reviewed in Hu *et al.* 1998, Hu *et al.* 2007). The half-life of Pb in bone ranges from 10 to 30 years, depending on the rate of

bone turnover, which in turn varies by type of bone and life stage (Rabinowitz 1991). In young children, continuous growth results in constant bone remodeling, and bone Pb is exchanged with blood Pb much more frequently than in adults (reviewed in Barbosa *et al.* 2005, Hu *et al.* 2007).

This evaluation focuses on the relationship between health effects and blood Pb levels because blood Pb is the most widely available measure of exposure, blood Pb reflects the equilibrium between current and past exposure, as described above, and numerous studies have reported an association between blood Pb levels and health outcomes. However, measuring Pb in one tissue at one point in time does not present a complete picture of either current or cumulative Pb exposure, and bone Pb reflects long-term stores of Pb in the body better than does blood Pb (reviewed in Barbosa *et al.* 2005, Hu *et al.* 2007); therefore, bone Pb data were also considered when available. Note that measuring bone Pb is expensive, requires specialized equipment that is not generally accessible, and requires study subjects to travel to the location of the measurement apparatus (K-x-ray fluorescence); thus, fewer Pb data are available for bone than for blood.

Before bans on Pb in paint, solder, and gasoline, environmental Pb levels in the United States were higher, so older adults accumulated more Pb as children than children do today. Average blood Pb levels in children 1-5 years of age have decreased 10-fold over the last 30 years, from 15.1 µg/dL in 1976-1980 to 1.51 µg/dL in 2007-2008 (geometric means; CDC 2007, 2011). This is clearly good news for current populations of children and represents a significant public health accomplishment. However, most U.S. adults who were born before 1980 had blood Pb levels >10 µg/dL during early childhood, so health effects in adults today may have been influenced by blood Pb levels >10 µg/dL that many individuals experienced earlier in life.

Keeping childhood blood Pb levels in mind, there are data on multiple health effects in adults for which studies report a significant relationship between concurrent blood Pb levels as adults and the health effect (e.g., elevated blood pressure, reduced kidney function, or decreases in specific measures of cognitive function). There is a considerable body of evidence that these health effects are associated with Pb exposure, and multiple studies report a significant association with concurrent blood Pb levels <10 µg/dL. Furthermore, the association with blood Pb is supported by the consistency of effects among epidemiological studies and biological coherence with animal data. It is well recognized that the role of early-life Pb exposure cannot be discriminated from the role of concurrent blood Pb without additional long-term studies. To eliminate the potential role of early-life blood Pb levels >10 µg/dL on health effects observed in adults with blood Pb levels <10 µg/dL, prospective studies (following a group over time) would be required in a group with blood Pb levels consistently <10 µg/dL from birth until measurement of the outcome of interest.

As described in **Section 1.2.2**, the NTP's conclusions were derived by evaluating data from epidemiological studies with a focus on blood Pb levels <10 µg/dL. The evidence discussed for specific health outcomes within each chapter varies by study design and type of analyses used to examine the relationship of the health outcome with blood Pb across the hundreds of studies evaluated. In some cases, studies examined only groups with blood Pb levels <10 µg/dL,

<5 µg/dL, or even lower, and the association of the health effect with the blood Pb level is clear. For example, Lanphear *et al.* (2000) reported that higher blood Pb levels were associated with lower academic performance in a cross-sectional study (examining one point in time) of 4,853 children 6-16 years of age from the NHANES III data set. When they analyzed only children with blood Pb <10 µg/dL (n=4,681) or <5 µg/dL (n=4,043), the association with blood Pb was still significant (p<0.001 for <10 µg/dL and <5 µg/dL). In other cases, studies reported a significant association between blood Pb and an effect in a group whose *mean* blood Pb level was <10 µg/dL (e.g., higher blood Pb levels were associated with higher blood pressure in 964 adults in the Baltimore Memory Study (Martin *et al.* 2006)). These analyses support an effect of a blood Pb level <10 µg/dL, but they do not exclude the possibility that individuals significantly above or below the mean blood Pb level are driving the effect, or that past exposure levels are driving the effect. Finally, some studies compared effects between two groups with higher and lower blood Pb levels. For example, Naicker *et al.* (2010) compared the effect of a blood Pb level ≥5 µg/dL with a blood Pb level <5 µg/dL on developmental markers of puberty in 13-year-old girls in South Africa (n=682) and found that a blood Pb level ≥5 µg/dL was significantly associated with delayed breast development, pubic hair development, and age of menarche.

1.4 Health Effects Evidence

1.4.1 NTP Conclusions

The NTP concludes that there is *sufficient* evidence for adverse health effects in children and adults at blood Pb levels <10 µg/dL, and <5 µg/dL as well (see **Table 1.1** for summary of effect by life stage at which the effect is identified). A major strength of the evidence supporting effects of low-level Pb comes from the consistency demonstrated by adverse effects associated with blood Pb <10 µg/dL across a wide range of health outcomes, across major physiological systems from reproductive to renal, among multiple groups, from studies using substantially different methods and techniques, and for health effects in both children and adults.

In children, there is *sufficient* evidence that blood Pb levels <5 µg/dL are associated with increased diagnosis of attention-related behavioral problems, greater incidence of problem behaviors, and decreased cognitive performance as indicated by (1) lower academic achievement, (2) decreased intelligence quotient (IQ), and (3) reductions in specific cognitive measures. There is also *limited* evidence that blood Pb <5 µg/dL is associated with delayed puberty and decreased kidney function in children ≥12 years of age. There is *sufficient* evidence that blood Pb levels <10 µg/dL in children are associated with delayed puberty and reduced postnatal growth. There is *limited* evidence that blood Pb levels <10 µg/dL are associated with elevated serum immunoglobulin E (IgE), which is a principal mediator of hypersensitivity; consistent with this effect, there is *limited* evidence that blood Pb levels <10 µg/dL are associated with changes to an IgE-related health effect, allergy diagnosed by skin prick test to common allergens. There is *inadequate* evidence of an association between blood Pb <10 µg/dL in children and other

Table 1.1: NTP conclusions on health effects of low-level Pb by life stage				
Life Stage	**Blood Pb Level**	**NTP Conclusion**	**Principal Health Effects**	**Bone Pb Evidence**
Children	<5 µg/dL	*Sufficient*	Decreased academic achievement, IQ, and specific cognitive measures; increased incidence of attention-related behaviors and problem behaviors	Tibia and dentin Pb are associated with attention-related behaviors, problem behaviors, and cognition.
		Limited	Delayed puberty and decreased kidney function in children ≥12 years of age	The one available study of bone Pb in children does not support an association with postnatal growth.
	<10 µg/dL	*Sufficient*	Delayed puberty, reduced postnatal growth, decreased IQ, and decreased hearing	No data
		Limited	Increased hypersensitivity/allergy by skin prick test to allergens and increased IgE* (not a health outcome)	No data
		Inadequate	Any age - asthma, eczema, nonallergy immune function, cardiovascular effects; <12 years of age - renal function	No data
Adults	<5 µg/dL	*Sufficient*	Decreased glomerular filtration rate; maternal blood Pb associated with reduced fetal growth	The one available study of bone Pb in the general population supports an association between bone Pb and decreased kidney function. Maternal bone Pb is associated with reduced fetal growth.
		Limited	Increased incidence of essential tremor	No data
	<10 µg/dL	*Sufficient*	Increased blood pressure, increased risk of hypertension, and increased incidence of essential tremor	The association between bone Pb and cardiovascular effects is more consistent than for blood Pb.
		Limited	Psychological effects, decreased cognitive function, decreased hearing, increased incidence of ALS, and increased cardiovascular-related mortality; maternal blood Pb associated with increased incidence of spontaneous abortion and preterm birth	The association between bone Pb and cognitive decline is more consistent than for blood Pb.
		Inadequate	Immune function, stillbirth, endocrine effects, birth defects, fertility or time to pregnancy**, and sperm parameters**	No data

Abbreviations: ALS, amyotrophic lateral sclerosis; IgE, immunoglobulin E; IQ, intelligence quotient

*Increased serum IgE is associated with hypersensitivity; however, as described in **Section 1.4.3**, increased IgE does not equate to disease.

The NTP concludes that there is inadequate evidence that blood Pb levels <10 µg/dL are associated with fertility, time to pregnancy, and sperm parameters; however, given the basis of the original nomination, the NTP evaluated the evidence that higher blood Pb levels (i.e., >10 µg/dL) are associated with reproductive and developmental effects, and those conclusions are discussed in **Section 1.4.6 and presented in **Table 1.2**.

allergic diseases, such as eczema or asthma. There is also *inadequate* evidence of an association between blood Pb <10 µg/dL and cardiovascular effects in children of any age, or renal function in children <12 years of age.

In adults, there is *sufficient* evidence that blood Pb levels <5 µg/dL are associated with decreased renal function and that blood Pb levels <10 µg/dL are associated with increased blood pressure and hypertension. There is *sufficient* evidence that maternal blood Pb levels <5 µg/dL are associated with reduced fetal growth and *limited* evidence that maternal blood Pb levels <10 µg/dL are associated with increased spontaneous abortion and preterm birth. There is *sufficient* evidence that blood Pb levels <10 µg/dL, and *limited* evidence that blood Pb levels <5 µg/dL, are associated with essential tremor in adults. There is also *limited* evidence for an association between blood Pb <10 µg/dL and increased cardiovascular-related mortality, decreased auditory function, the neurodegenerative disease amyotrophic lateral sclerosis (ALS), and decreases in specific measures of cognitive function in older adults. The NTP conclusions of associations between blood Pb levels <10 µg/dL in adults and health effects cannot completely eliminate the potential contributing effects of early-life blood Pb levels, as discussed in **Section 1.3**.

Although the relationship between many health effects and bone Pb as a measure of exposure has not been examined, the data support the importance of cumulative Pb exposure on cardiovascular effects of Pb in adults, as well as neurocognitive decline in adults, because the association between Pb and these endpoints is more consistent for bone Pb than for blood Pb.

1.4.2 Neurological Effects

The NTP concludes that there is *sufficient* evidence that blood Pb levels <5 µg/dL are associated with adverse neurological effects in children and *limited* evidence that blood Pb levels <10 µg/dL are associated with adverse neurological effects in adults (see **Table 1.2** for summary of effects).

Unlike the data set for most other health effect areas, there are a number of prospective studies of neurological effects that include measures of prenatal exposure (either maternal blood or umbilical cord blood Pb levels). These prospective studies provide *limited* evidence that prenatal exposure to blood Pb levels <5 µg/dL is associated with decreases in measures of general and specific cognitive function evaluated in children. There is also *limited* evidence that prenatal exposure to blood Pb levels <10 µg/dL is associated with decreased IQ, increased incidence of attention-related behaviors and antisocial behavior problems, and decreased hearing measured in children. However, conclusions about effects of prenatal Pb exposure for outcomes evaluated as children are complicated by the high degree of correlation between prenatal and childhood blood Pb levels and as described below, blood Pb levels during childhood are also associated with these effects.

In children, there is *sufficient* evidence that blood Pb levels <5 µg/dL are associated with decreases in broad based and specific indices of cognitive function and an increase in attention-

Table 1.2: NTP conclusions on health effects of low-level Pb by major health effect areas

Health Area	Population or Exposure Window		NTP Conclusion	Principal Health Effects	Blood Pb Evidence	Bone Pb Evidence
Neurological	Prenatal		*Limited*	Decrease in measures of cognitive function	Yes, <5 µg/dL	No data
			Limited	Decreased IQ, increased incidence of attention-related and problem behaviors, decreased hearing	Yes, <10 µg/dL	No data
	Children		*Sufficient*	Decreased academic achievement, IQ, and specific cognitive measures; increased incidence of attention-related and problem behaviors	Yes, <5 µg/dL	Tibia and dentin Pb are associated with attention, behavior, and cognition.
			Sufficient	Decreased hearing	Yes, <10 µg/dL	No data
	Adults		*Sufficient*	Increased incidence of essential tremor	Yes, <10 µg/dL	No data
			Limited	Psychiatric effects, decreased hearing, decreased cognitive function, increased incidence of ALS	Yes, <10 µg/dL	The association between bone Pb and cognitive decline is more consistent than blood.
			Limited	Increased incidence of essential tremor	Yes, <5 µg/dL	
Immune	Children		*Limited*	Increased hypersensitivity/allergy by skin prick test to common allergens and IgE* (not a health outcome)	Yes, <10 µg/dL	No data
			Inadequate	Asthma, eczema	Unclear	No data
	Adults		*Inadequate*	—	Unclear	No data
Cardiovascular	Children		*Inadequate*	—	Unclear	No data
	Adults		*Sufficient*	Increased blood pressure and increased risk of hypertension	Yes, <10 µg/dL	The association between bone Pb and cardiovascular effects is more consistent than blood.
			Limited	Increased cardiovascular-related mortality and ECG abnormalities	Yes, <10 µg/dL	
Renal	Children <12 years old		*Inadequate*	—	Unclear	No data
	Children ≥12 years old		*Limited*	Decreased glomerular filtration rate	Yes, <5 µg/dL	No data
	Adults		*Sufficient*	Decreased glomerular filtration rate	Yes, <5 µg/dL	Yes, one study
Reproductive and Developmental	Prenatal		*Limited*	Reduced postnatal growth	Yes, <10 µg/dL	No data
	Children		*Sufficient*	Delayed puberty, reduced postnatal growth	Yes, <10 µg/dL	One study does not support effects of bone Pb on growth.
			Limited	Delayed puberty	Yes, <5 µg/dL	
	Adults	Women	*Sufficient*	Reduced fetal growth	Yes, <5 µg/dL	Maternal tibia Pb is associated
			Limited	Increase in spontaneous abortion and preterm birth	Yes, <10 µg/dL	No data
		Men	*Sufficient*	Adverse changes in sperm parameters and increased time to pregnancy	Yes, ≥15-20 µg/dL	No data
			Limited	Decreased fertility	Yes, ≥10 µg/dL	No data
			Limited	Increased spontaneous abortion	Yes, >31 µg/dL	No data
		Adults	*Inadequate*	Stillbirth, endocrine effects, birth defects	Unclear	No data

Abbreviations: ALS, amyotrophic lateral sclerosis; ECG, electrocardiography; IgE, immunoglobulin E; IQ, intelligence quotient.
*Increased serum IgE is associated with hypersensitivity; however, as described in **Section 1.4.3**, increased IgE does not equate to disease.

related behavioral problems and antisocial behavioral problems. The association between blood Pb and decreased IQ has been demonstrated in multiple prospective studies of children with blood Pb levels <10 µg/dL, pooled analyses that reported effects with peak blood Pb levels <7.5 µg/dL (Lanphear *et al.* 2005), and multiple cross-sectional studies that reported effects with mean blood Pb levels <5 µg/dL. Lower levels of academic achievement, as determined by class rank and achievement tests, have been reported in multiple prospective and cross-sectional studies of children with blood Pb <5 µg/dL. An association between blood Pb <5 µg/dL and decreases in specific measures of cognitive function has been demonstrated in prospective and cross-sectional studies using a wide range of tests to assess cognitive function. Increases in attention-related and problem behaviors are consistently reported in studies with mean blood Pb levels <5 µg/dL. The NTP concludes that blood Pb is associated with attention-related behaviors rather than attention deficit hyperactivity disorder (ADHD) alone because (1) this broad term more accurately reflects the range of Pb-associated behavioral effects in the area of attention, of which ADHD is one example on the more severe end of the spectrum, and (2) determination of ADHD in children from available studies are not as precise as an ADHD diagnosis by trained clinicians using specific *DSM-IV-TR* criteria. There is *sufficient* evidence that blood Pb levels <10 µg/dL in children are associated with decreased auditory acuity. Multiple cross-sectional studies reported hearing loss, as indicated by higher hearing thresholds and increased latency of brainstem auditory evoked potentials (BAEPs), in children with blood Pb levels <10 µg/dL.

In adults, there is *limited* evidence that blood Pb levels <10 µg/dL are associated with psychiatric outcomes (including anxiety and depression), decreased auditory function, ALS, and decreases in specific measures of cognitive function in older adults. There is *sufficient* evidence that blood Pb levels <10 µg/dL are associated with essential tremor in adults, and limited evidence for blood Pb levels <5 µg/dL. Associations with decreases in cognitive function in adults are more consistent for bone Pb than for blood Pb, suggesting a role for cumulative Pb exposure.

1.4.3 Immune Effects

The NTP concludes that there is *limited* evidence that blood Pb levels <10µg/dL are associated with adverse immune effects in children and that there is *inadequate* evidence in adults (see **Table 1.2**).

In children, there is *limited* evidence that blood Pb levels <10 µg/dL are associated with changes to an immune-related health outcome such as allergy or increased hypersensitivity. There is also *limited* evidence that blood Pb levels <10 µg/dL are associated with elevated serum IgE levels. Five studies of groups with mean blood Pb levels of 10 µg/dL and below support the relationship between blood Pb and increased serum IgE. Two of these studies reported an association at blood Pb levels of ≥10 µg/dL rather than <10 µg/dL, and only one of the remaining studies adjusted for age, a particularly important confounder in analyses of IgE in children. Although increases in serum levels of total IgE are not definitive indicators of allergic disease, elevated levels of IgE are primary mediators of hypersensitivity associated with sensitization and allergic disease. Therefore, the studies demonstrating Pb-related increases in

IgE suggest a link to hypersensitivity and support more definitive data such as a prospective study that found blood Pb levels <10 µg/dL were associated with increased hypersensitivity (or allergy by skin prick testing) in children. These data support the conclusion of *limited* evidence that increased hypersensitivity responses or allergy are associated with blood Pb levels <10 µg/dL in children; however, there is *inadequate* evidence of an association between blood Pb and other allergic diseases such as eczema or asthma.

There is *inadequate* evidence in adults to address the potential association between blood Pb <10 µg/dL and IgE, allergy, eczema, or asthma. Few studies have investigated the relationship between immune function and Pb in humans, and most studies reported general observational markers of immunity rather than function. There is *inadequate* evidence that blood Pb levels <10 µg/dL are associated with observational immune effects such as altered lymphocyte counts or serum levels of IgG, IgM, or IgA in the blood of children or adults, because few studies have examined the lower exposure level and the available data are inconsistent. There is also *inadequate* evidence that blood Pb levels <10 µg/dL are associated with changes in immune function other than hypersensitivity, because few studies have examined immune function at lower blood Pb levels.

Bone Pb levels may be particularly relevant for cells of the immune system and immune function. All of the white blood cells or leukocytes that develop after birth are derived from progenitor cells in the bone marrow. Unfortunately, very few studies of immune effects have measured exposure other than blood Pb; therefore, the relative importance of blood or bone Pb levels for immune effects of Pb is unknown.

1.4.4 Cardiovascular Effects

The NTP concludes that there is *sufficient* evidence that blood Pb levels <10 µg/dL in adults are associated with adverse effects on cardiovascular function and that there is *inadequate* evidence to evaluate cardiovascular effects in children (see **Table 1.2** for summary of effects).

There is *sufficient* evidence of a bone Pb-related increase in the risk of hypertension and increases in blood pressure in adults. Two prospective studies and five cross-sectional studies support a significant association between bone Pb and blood pressure or hypertension in groups with blood Pb levels <10 µg/dL. Studies show less consistent associations between blood Pb and blood pressure or hypertension than for bone Pb; however, most of the recent studies with mean blood Pb levels <5 µg/dL found significant associations between concurrent blood Pb levels and increased blood pressure. There is *sufficient* evidence that blood Pb levels <10 µg/dL increase the risk of hypertension during pregnancy, supported by one prospective study and five cross-sectional studies with blood Pb levels during pregnancy <10 µg/dL. There is *limited* evidence of increased risk of cardiovascular mortality associated with blood Pb levels <10 µg/dL. An association between increased cardiovascular mortality and blood Pb is supported by three prospective studies (two of which used the same NHANES III sample) but is not supported by two other prospective studies. One of the studies that did not find an association with blood Pb (at a mean blood Pb level of 5.6 µg/dL) reported a significant association between bone Pb levels and increased cardiovascular mortality. There is *limited*

evidence for Pb effects on other cardiovascular outcomes, including electrocardiography (ECG) abnormalities and clinical cardiovascular disease primarily due to lack of replication studies. Chronic Pb exposure appears to be more critical than current Pb exposure, as shown by more consistent associations between chronic cardiovascular effects and bone Pb than for blood Pb. Studies support an association with concurrent blood Pb levels; however, the potential effect of early-life blood Pb levels on cardiovascular outcomes in adults cannot be discriminated from the effect of concurrent blood Pb levels without additional prospective studies in a population for which blood Pb levels remain consistently below 10µg/dL from birth until evaluation of the various cardiovascular outcomes as described in **Section 1.3**. There is *inadequate* evidence for Pb effects on heart rate variability, due to a lack of replicated studies.

There is *inadequate* evidence to assess whether children or menopausal women present a sensitive life stage for cardiovascular effects of Pb. No prospective studies have followed children with early-life Pb measures and evaluated cardiovascular health in adulthood. During periods of bone demineralization such as menopause and with osteoporosis, Pb stored in bone may enter the blood stream at a higher rate, increasing circulating Pb levels; for example, increased blood Pb levels have been demonstrated in women after menopause in several studies (e.g., Silbergeld *et al.* 1988, Symanski and Hertz-Picciotto 1995, Webber *et al.* 1995, Korrick *et al.* 2002). Too few studies have examined Pb-related cardiovascular health risks in postmenopausal women to enable conclusions.

Although hypertension can contribute to adverse renal effects, and kidney dysfunction can contribute to increased blood pressure, effects are considered separately in this evaluation because most studies examined one outcome or the other, rather than testing both systems comprehensively.

1.4.5 Renal Effects

The NTP concludes that there is *sufficient* evidence that blood Pb levels <5 µg/dL are associated with adverse renal effects in adults (see **Table 1.2** for summary of effects). There is *limited* evidence that blood Pb levels <5 µg/dL are associated with adverse renal effects in children ≥12 years of age, and the current evidence is *inadequate* to conclude that blood Pb <10 µg/dL is associated with renal effects in children <12 years of age.

There is *sufficient* evidence that blood Pb levels <5 µg/dL are associated with adverse effects on kidney function in adults. Most of the 13 epidemiological studies of the general population reported blood Pb levels <10 µg/dL are associated with (1) increased risk of chronic kidney disease (CKD), and (2) decreases in the estimated glomerular filtration rate (eGFR) and creatinine clearance, markers of kidney function. The associations are typically stronger in studies of groups with hypertension or diabetes. Few studies have examined other markers of Pb exposure, such as bone Pb; therefore, it is unknown whether blood or bone Pb levels would be a better measure of exposure for kidney effects related to Pb. Epidemiological data from the general population support an association with concurrent blood Pb levels in adults; however, the potential effect of early-life blood Pb levels on kidney function in adults cannot be discriminated from the effect of concurrent blood Pb levels without additional prospective

studies in a group for which blood Pb levels remain consistently below 10µg/dL from birth until evaluation of kidney function as described in **Section 1.3**.

There is *inadequate* evidence to address the potential association between blood Pb levels <10 µg/dL in children <12 years of age and impaired kidney function, because results are inconsistent and available studies of kidney function in young children are less reliable in general because tests of kidney function lack clear predictive value in this age group. There is *limited* evidence that blood Pb levels <5 µg/dL are associated with adverse effects on kidney function in children ≥12 years of age. This conclusion is based on one study of NHANES data, which reported effects in children ≥12 years of age that are consistent with reduced eGFR reported in adults in several NHANES studies.

1.4.6 Reproduction and Developmental Effects

The NTP concludes that there is *sufficient* evidence that blood Pb levels <10 µg/dL are associated with adverse health effects on development in children and that blood Pb levels <5 µg/dL are associated with adverse health effects on reproduction in adult women (see **Table 1.2** for summary of effects).

Because most data on reproductive effects come from studies of occupational exposure, many of the available studies are for blood Pb levels >10 µg/dL. For this reason, and because the original nomination focused on reproductive and developmental effects, the evaluation of health effects in this area includes higher blood Pb levels, unlike other sections of this document. Consideration of these higher blood Pb levels resulted in several conclusions for Pb-related reproductive effects in men but did not affect the conclusions for women or children.

Unlike the data for most other health effect areas, a number of prospective studies of developmental effects have included prenatal measures of exposure (either maternal blood or umbilical cord blood). These prospective studies provide *limited* evidence that prenatal exposure to blood Pb levels <10 µg/dL is associated with reduced postnatal growth in children. Conclusions about effects of prenatal Pb exposure in children are complicated because blood Pb levels <10 µg/dL during childhood are also associated with reduced postnatal growth, and prenatal Pb levels are highly correlated with childhood Pb levels.

In children, there is *sufficient* evidence that blood Pb levels <10 µg/dL are associated with delayed puberty and *limited* evidence for this effect at blood Pb levels <5 µg/dL. Nine studies reported that concurrent blood Pb levels <10 µg/dL in children are associated with delayed puberty. There is *sufficient* evidence that blood Pb levels <10 µg/dL are associated with decreased postnatal growth. Numerous cross-sectional studies, including studies with large sample sizes such as the NHANES data sets, reported that concurrent blood Pb <10 µg/dL in children is associated with reduced head circumference, height, or other indicators of growth.

In adults, there is *sufficient* evidence that maternal blood Pb levels <5 µg/dL are associated with reduced fetal growth or lower birth weight. Three prospective studies with maternal blood Pb data during pregnancy, a large retrospective study (examining medical history) of >43,000

mother-infant pairs with a mean maternal blood Pb level of 2.1 μg/dL, and several cross-sectional studies of Pb levels in maternal or cord blood at delivery support an association between higher blood Pb and reduced fetal growth at mean blood Pb levels from 1 to 10 μg/dL. Although maternal or paternal bone Pb data are not available in most studies of reproductive health outcomes, a set of studies of a single group reported that higher maternal bone Pb is related to lower fetal growth. There is also *limited* evidence that maternal blood Pb levels <10 μg/dL are associated with preterm birth and spontaneous abortion. Although several prospective studies reported an association between maternal blood Pb and preterm birth, the conclusion of *limited* evidence is due to inconsistent results and a retrospective study with a large cohort of >43,000 mother-infant pairs not finding an association between maternal blood Pb levels and preterm birth. The conclusion of *limited* evidence for an association with spontaneous abortion is based primarily on the strength of a single prospective nested case-control study in women, with additional support provided by occupational studies that reported an association with Pb exposure but lacked blood Pb measurements. In men, there is *inadequate* evidence that blood Pb levels <10 μg/dL are associated with effects on reproduction.

In men there is *sufficient* evidence that blood Pb levels ≥15 μg/dL are associated with adverse effects on sperm or semen and that blood Pb levels ≥20 μg/dL are associated with delayed conception time. Decreases in sperm count, density, and concentration have been reported in multiple retrospective and cross-sectional occupational studies of men with mean blood Pb levels from 15 to 68 μg/dL. Four studies reported increased time to pregnancy in women whose male partners had blood Pb levels of 20-40 μg/dL. A single retrospective occupational study reported increased risk of infertility among men with blood Pb levels ≥10 μg/dL, and the consistency of this observation with other studies reporting effects on time to pregnancy at higher blood Pb levels supports a conclusion of *limited* evidence that blood Pb levels ≥10 μg/dL in men are associated with other measures of reduced fertility. There is also *limited* evidence that paternal blood Pb levels >31 μg/dL are associated with spontaneous abortion, based primarily on the strength of a single retrospective nested case-control study in men, with additional support provided by occupational studies that reported an association with Pb exposure but lacked blood Pb measurements.

1.5 Future Research

There are robust data and *sufficient* evidence that blood Pb levels <10 μg/dL in children and adults are associated with adverse health effects across a wide range of health outcomes, as described above. Over time, epidemiological studies have provided data to support health effects at lower and lower blood Pb levels, particularly in children. Prospective studies in children better address the lower limits of Pb exposure associated with health effects because they focus on children whose blood Pb levels remain <10 μg/dL or <5 μg/dL with certainty throughout their lifetime. Studies of health effects in adults cannot eliminate the potential effects of early-life blood Pb levels on health effects observed as adults. This is particularly important in an evaluation of the health effects of blood Pb levels <10 μg/dL because older adults were likely to have had blood Pb levels >10 μg/dL as children (see discussion in

Section 1.3), compared with only 0.8% of children with confirmed blood Pb levels >10 µg/dL in 2008.

Clarification of the effects of early-life blood Pb levels relative to the effects of concurrent blood Pb levels remains a significant issue for evaluating Pb-related health effects in adults. Epidemiological data from adults support an association between concurrent blood Pb levels <5 µg/dL and decreased renal function and between concurrent blood Pb levels <10 µg/dL and increased blood pressure and hypertension. Future research should be directed at clarifying the extent to which early life exposure (e.g., blood Pb levels >10 µg/dL) contribute to health effects observed in adults. Long-term prospective studies in a group for which blood Pb levels remain consistently <10 µg/dL from birth until the outcome of interest is measured would take one step in this direction by eliminating the potential role of early-life blood Pb levels >10 µg/dL on health effects observed in adults with concurrent blood Pb levels <10 µg/dL.

2.0 METHODS

The NTP's conclusions on health effects of low-level Pb are based on evaluation of data from epidemiological studies with a focus on blood Pb levels <10 µg/dL. The methodological approach began with a statement of the key questions addressed by this evaluation. The general approach for developing the NTP's conclusions on evidence of an association between blood Pb levels <10 µg/dL and specific health effects is described below, along with the format and definitions used throughout the document. The structure of appendix tables summarizing the relevant literature for each health effect area is also described below. The NTP considered several recent government evaluations of the health effects of Pb as authoritative sources to supplement a review of the primary epidemiological literature, and these documents are briefly described in this section. The NTP also used independent subject matter experts as technical advisors to provide scientific input and to review pre-public release drafts of each chapter summarizing the evidence for health effects associated with low-level Pb, as well as the appendices and background exposure section. The literature search strategy and details of the peer-review process are also described below.

2.1 Key Questions

What is the evidence that adverse health effects are associated with blood Pb <10 µg/dL?

- What reproductive, developmental, neurological, immune, cardiovascular, and renal health effects are associated with blood Pb levels <10 µg/dL?
- What is the blood Pb level associated with a given health effect (i.e., <10 µg/dL or <5 µg/dL)?
- At which life stages (childhood or adulthood) is the effect identified?
- Are there data to evaluate the association between bone Pb and the health effect, and how does the association to this biomarker of Pb exposure compare to the association with blood Pb?

2.2 Approach to Develop Health Effects Conclusions

Conclusions in the NTP evaluation of Pb-related health effects in humans associated with low-level Pb were derived by evaluating the data from epidemiological studies with a focus on blood Pb levels <10 µg/dL. The evaluation includes a review of the primary epidemiological literature, and these studies formed the basis for the NTP conclusions. The quality of individual studies was considered in reaching health effects conclusions, including consideration of known confounders, appropriateness of the method of diagnosis, strength of the study design, and the sample size. General strengths and limitations of study designs were considered when developing conclusions, with prospective studies providing stronger evidence than cross-sectional or case-control studies. Consistency of effects across the body of evidence and important factors such as the number of studies, exposure levels, biological plausibility, and support from the animal literature were all assessed when developing the NTP conclusions.

Draft NTP conclusions were evaluated for consistency with health effect conclusions from recent government evaluations considered authoritative sources: the U.S. EPA 2006 Air Quality Criteria Document (AQCD) for Lead (U.S. EPA 2006) and the ATSDR 2007 Toxicological Profile for Lead (ATSDR 2007) (see **Section 2.4** for discussion). Technical advisors with relevant subject matter expertise served as another authoritative source (see **Section 2.4.4** for details). Technical advisors were asked to critically evaluate every one of the NTP's conclusions regarding the potential for adverse health effects to occur at blood Pb levels <10 µg/dL and to determine whether the science cited was technically correct, clearly stated, and supported the NTP's conclusions in pre-public release drafts of each chapter addressing specific health effect areas. As described in **Section 2.6** and **Peer-Review of the Draft NTP Monograph on Health Effects of Low-level Lead**, a draft version of the Monograph was released for public comment and peer review by an expert panel of *ad hoc* reviewers. Written public comments and comments from peer reviewers were considered during finalization of the document.

Studies were evaluated for evidence that low-level Pb is associated with neurological, immunological, cardiovascular, renal, and/or reproductive and developmental effects. These health effects areas were selected because there is a relatively large database of human studies in each area. The NTP considered four possible conclusions for specific health effects within each area:

> **Sufficient Evidence of an Association**: an association is observed between the exposure and health outcome in studies in which chance, bias, and confounding could be ruled out with reasonable confidence.
>
> **Limited Evidence of an Association**: an association is observed between the exposure and health outcome in studies in which chance, bias, and confounding could not be ruled out with reasonable confidence.
>
> **Inadequate Evidence of an Association**: the available studies are insufficient in quality, consistency, or statistical power to permit a conclusion regarding the presence of absence of an association between exposure and health outcome, or no data in humans are available.
>
> **Evidence of No Association:** several adequate studies covering the full range of levels of exposure that humans are known to encounter (in this case limited to blood Pb levels <10 µg/dL) are mutually consistent in not showing an association between exposure to the agent and any studied endpoint.

The discussion of each health effect begins with a statement of the NTP's conclusion regarding whether the specific effect is associated with a blood Pb level <10 µg/dL or <5 µg/dL and the age group in which it is or is not identified (childhood or adulthood), as well as the timing of exposure associated with the effect (prenatal, childhood, concurrent) when available. Then key data and principal studies considered in developing the NTP's conclusions are then discussed in detail. Each section concludes with a summary discussing each health effect, describing

experimental animal data that relate to the human data, and stating the basis for the NTP's conclusions.

For the purposes of this evaluation, "children" refers to individuals <18 years of age unless otherwise specified. In addition to the blood Pb level of <10 µg/dL, a lower effect level of <5 µg/dL was also selected because it is commonly used in epidemiological studies to categorize health effect data by exposure levels; therefore, data are often available to evaluate health effects for groups above and below this value as well. Findings described in the text as having an "association" or "significant association" reflect a statistically significant result with a p-value <0.05 unless otherwise indicated.

2.3 Appendices of Studies Considered

The information to support the NTP's conclusions for individual health effects is presented in each chapter. Human studies from groups with low-level Pb exposure that were considered in developing the conclusions are also abstracted for further reference and are included in separate appendices for each health effect area.

Each appendix table includes the following column headings:

Description: study design, reference, and geographic location
Population: sample size, description, years of study, and percent male
Age: mean age and standard deviation of the subjects
Blood Pb: mean blood Pb level and standard deviation (in µg/dL)
Outcomes: health effects assessed
Statistical: methods used and cofactors included in analyses
Findings: summary of results (bolded if statistical significance tests had a p-value <0.05)
Observed effect: conclusion (Effect/No Effect/Equivocal) and description

Potential overlap of subjects in multiple publications from the same epidemiological study is indicated in the first column of each appendix. These studies were not considered as independent findings to be evaluated in developing the NTP's conclusions.

The grouping of studies within each appendix table varied by health effects considered:

Appendix A. Neurological Effects: no grouping, meta-analyses shaded
Appendix B. Immune Effects: grouped by low (<15 µg/dL) and high (>15 µg/dL) exposure
Appendix C. Cardiovascular Effects: grouped by outcome, meta-analyses shaded
Appendix D. Renal Effects: no grouping
Appendix E. Reproductive and Developmental Effects: grouped by outcome

Within each grouping, studies are listed alphabetically by first author and then chronologically by publication date. For the appendix tables grouped by outcome, if a publication contained results that applied to more than one group, results specific to each outcome group were included.

The NTP's conclusions are based on the evidence from human studies with blood Pb levels of <10 μg/dL and therefore the abstracted studies in the appendices are mainly those with a mean blood Pb level of <10 μg/dL. However, studies with data reflecting mean exposure levels up to 15 μg/dL were also included so that effects at and around 10 μg/dL were not missed during the evaluation. Reproductive effects in studies with mean blood Pb levels >15 μg/dL were included in the evaluation because the data set of human studies on these effects associated with lower blood Pb levels is limited. For this reason, and because the original nomination focused on reproductive and developmental effects, the evaluation of health effects in this area includes higher blood Pb levels. The immunological effects database was adequate to make conclusions on several effects at blood Pb levels <10 μg/dL; however, because the NTP makes limited reference to studies in humans at higher blood Pb levels, Appendix B also includes human studies with higher blood Pb levels (i.e., >15 μg/dL).

2.4 Authoritative Sources Considered

Recent government evaluations of the health effects of Pb include the U.S. EPA 2006 AQCD for Lead (U.S. EPA 2006), the ATSDR 2007 Toxicological Profile for Lead (ATSDR 2007), and the CDC's Advisory Committee on Childhood Lead Poisoning Prevention (ACCLPP) Reports, such as the 2010 Guidelines for the Identification and Management of Lead Exposure in Pregnant and Lactating Women (CDC 2010). The NTP made extensive use of these evaluations in its assessment, especially the EPA's 2006 AQCD for Lead (U.S. EPA 2006) because it underwent extensive external public peer review. NTP considered the conclusions and data summaries from the EPA and ATSDR documents. In general, NTP concurred with the conclusions and agreed that the data support them. Differences between the NTP's conclusions and the 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) and the EPA's 2006 AQCD (U.S. EPA 2006) are identified for specific endpoints in this document. The database of studies on health effects in humans is supported by an equally large body of experimental animal studies. In this document, the experimental animal data are considered when relevant to reaching conclusions primarily based on the human literature. The reader is referred to the U.S. EPA AQCD for Lead (U.S. EPA 2006) and ATSDR Toxicological Profile for Lead (ATSDR 2007) for more in-depth reviews of the animal data.

2.4.1 U.S. EPA 2006 Air Quality Criteria Document (AQCD) for Lead

The EPA's AQCD is an exhaustive review and assessment (>1,200 pages with an additional 900 pages of tables and other annex material) of the scientific information related to human health and ecological effects associated with Pb in ambient air (U.S. EPA 2006). The EPA's AQCDs are published periodically (the latest draft document, released February of 2012, updates the review with literature published since the U.S. EPA 2006 AQCD for Lead (U.S. EPA 2006)) to provide key scientific assessment of evidence to support periodic review of the current Pb National Ambient Air Quality Standards. The 2006 EPA AQCD for Lead (U.S. EPA 2006) is an extensively reviewed document that was subject to public comment and review by the Clean Air Scientific Advisory Committee in a series of public meetings. Because EPA is in the process of revising the AQCD, the 2012 Integrated Science Assessment for Lead (U.S. EPA 2012) is available only as a draft at this time.

2.4.2 ATSDR 2007 Toxicological Profile for Lead

The 2007 Toxicological Profile for Lead (ATSDR 2007) is a comprehensive evaluation of the available toxicological and epidemiological data on Pb. The toxicological profile is organized around a public health statement summarizing the toxicological and adverse health effects for Pb. ATSDR's peer-review process for their toxicological profiles includes release for public comment and a peer review by a panel of experts.

2.4.3 CDC Lead Panel Documents

CDC's fifth revision of the statement on preventing Pb poisoning in young children includes a companion document developed by the ACCLPP that reviews the scientific evidence for adverse health effects in children at blood Pb levels <10 µg/dL. The committee concluded that the "overall weight of the evidence supports an inverse (negative) association between BLLs [blood lead levels] <10 µg/dL and the cognitive function in children" (CDC 2005). The report focuses primarily on cognitive function, but the committee also concluded that additional health effects (e.g., other neurological functions, stature, sexual maturation) were associated with blood Pb levels <10 µg/dL in children.

The ACCLPP has also prepared a draft report providing Guidelines for the Identification and Management of Lead Exposure in Pregnant and Lactating Women (CDC 2010). The report provides "practical considerations regarding preventing lead exposure during pregnancy, assessment and blood lead testing during pregnancy, medical and environmental management to reduce fetal exposure, breastfeeding and follow-up of infants and children exposed to lead in utero." The document summarizes the evidence from human studies through 2008 for health effects of Pb in pregnant women and the developing child (concentrating on exposure during gestation and from breastfeeding) and provides guidance for clinicians.

2.4.4 Technical Advisors

The primary mechanism for obtaining scientific input during development of the draft NTP Monograph on Health Effects of Low-Level Pb was through technical advisors (see **Contributors** for list of technical advisors). Technical advisors with relevant subject matter expertise were asked to provide input on issues of scientific complexity, adequacy of the literature review, and overall presentation of a pre-public release version of the draft NTP monograph. These advisors critically evaluated each of the NTP's health effects conclusions and the basis for those conclusions, as well as the appendices and the background exposure section. Individuals who served as technical advisors were screened for potential conflict of interest.

2.5 Literature Search Strategy

The 2006 EPA AQCD for Lead (U.S. EPA 2006) and the 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) were screened for citations on health effects assessed at low-level Pb exposure. The NTP's conclusions are based on the evidence from human studies with blood Pb levels of <10 µg/dL with data reflecting mean exposure levels up to 15 µg/dL also considered so that effects at and around 10 µg/dL were not missed during the evaluation. Primary literature

searches in MEDLINE®, Web of Science, Scopus, Embase, and TOXNET were conducted on March 1-5, 2010, to identify relevant studies published subsequent to the 2006 EPA and 2007 ATSDR documents. Search terms included the following MeSH subject headings: lead or lead poisoning; "diseases category" or "anatomy category" for health effects; and humans[mh or Medical Subject Headings in MEDLINE] or epidemiology[sh or SubHeadings in MEDLINE] or epidemiologic studies[mh] or age groups[mh] for limiting to human studies.

Because of the heteronym nature of the term "lead," text word searching used four approaches: (1) searched for "lead" in title; (2) used various combinations to focus on low-level exposure: "low lead" or "low blood lead" or "lower lead" or "lower blood lead" or "low level" or "low levels" or "lower level" or "lower levels" or "lead level" or "lead levels" or "low dose" or "lead induced" or "lead intake" or "blood lead"; (3) combined "lead" with heavy metals or cadmium or mercury or arsenic; and (4) when necessary, excluded "lead to" or "leads to" from search results. For databases that allowed proximity searching, "lead" and "low or lower" were required to be in the same sentence. This strategy would retrieve articles such as "low cadmium and lead levels" or "low blood and urine lead levels" or "lower concentrations of lead in the blood." Text words used to retrieve human studies included human(s), resident(s), inhabitant(s), population, people, subject(s), patient(s), case(s), women, men, girls, boys, parent(s), mother(s), father(s), adult(s), child, children, childhood, adolescent(s), infant(s), toddler(s), newborn(s), occupation(al), work, workplace, worker(s), employee(s), laborer(s), and staff.

An updated search was performed September 12-15, 2011, to identify any additional references published since the last search. Technical advisors who were involved in the review of the draft document (see below) were also asked to identify relevant studies. In addition, NTP published a *Federal Register* notice regarding the low-level Pb evaluation, inviting submission of information about recently published/in-press studies that might be relevant for consideration in the evaluation (75 FR 51815).

2.6 Peer-Review Process

Peer review of the draft NTP Monograph on Health Effects of Low-Level Lead was conducted by an expert panel of *ad hoc* reviewers with relevant scientific background (i.e., expertise in Pb or metals related to reproductive and developmental toxicology, neurotoxicology, immunotoxicology, cardiovascular toxicology, renal toxicology, and exposure) at a public meeting held November 17-18, 2011 in Research Triangle Park, NC (see **Peer Review of the Draft NTP Monograph on Health Effects of Low-level** Lead for list of panel members). The selection of panel members and conduct of the peer review were performed in accordance with the Federal Advisory Committee Act and Federal policies and regulations. The panel was charged to determine whether the science cited in the draft NTP Monograph on Low-Level Lead was technically correct, was clearly stated, and supported NTP's conclusions regarding the potential for adverse health effects to occur at blood Pb levels <10 µg/dL. Public comments received as part of the NTP's evaluation of health effects of low-level Pb, meeting minutes, and other materials from the peer-review meeting are available at

http://ntp.niehs.nih.gov/go/37090. Written public comments and comments from peer reviewers were considered during finalization of the document.

3.0 EXPOSURE

Studies of health effects of Pb in humans commonly use one of several biomarkers to reflect the level of Pb exposure in an individual. The overwhelming majority of studies measure whole-blood Pb because blood samples are routinely collected and stored in large epidemiological studies; furthermore, the methods for measuring Pb in whole blood are widespread and extensively validated. Bone Pb is more likely to reflect cumulative exposure but must be measured by specialized equipment and requires measurements to be made on subjects present at a research clinic. Measures of Pb in urine and hair have been used in some studies, but how well they reflect the body burden of Pb is less clear.

Pb is ubiquitous in the environment, but the level of exposure to Pb that individuals experience can vary and depends on many factors, including occupation, geography, and life stage. This chapter briefly discusses common routes of exposure to Pb and associated factors that may affect the risk of exposure.

The NTP made extensive use of recent government evaluations of Pb exposure and associated health effects in developing the current assessment. The EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) both contain extensive review and discussion of Pb exposure, with the AQCD document particularly focusing on Pb in air. The NTP also used two CDC documents focused on particularly vulnerable groups: the 2010 Guidelines for the Identification and Management of Lead Exposure in Pregnant and Lactating Women and the 2005 Preventing Lead Poisoning in Young Children report (CDC 2005b, 2010). The EPA is in the process of revising the AQCD and has released an external review draft that also includes extensive discussion of Pb exposures (U.S. EPA 2012).

3.1 What Does It Mean to Refer to Blood Pb <10 µg/dL?

This evaluation focuses on the relationship between health effects and blood Pb levels <10 µg/dL because (1) whole-blood Pb is the most widely available measure of exposure, (2) blood Pb reflects an equilibrium between current environmental Pb exposures and Pb stored in bone from prior exposures, and (3) numerous studies have reported an association between blood Pb levels and health effects.

However, measuring Pb in one tissue at one point in time does not present a complete picture of either current or cumulative Pb exposure. Bone Pb is considered superior to blood Pb in reflecting the long-term stores of Pb in the body (e.g., Rabinowitz 1991, reviewed in Barbosa *et al.* 2005, Hu *et al.* 2007). When available, bone Pb data were also considered in this evaluation. However, measuring bone Pb is expensive and requires subjects to travel the location of the specialized measurement apparatus (K-x-ray fluorescence). A number of authors have hypothesized that blood Pb may provide a better measure of Pb exposure in children or other subjects with active bone remodeling (see reviews by Barbosa *et al.* 2005, Hu *et al.* 2007). However, few studies in children have examined the usefulness of bone Pb data as a measure

of exposure in children to test this hypothesis, other than studies that reported Pb levels in shed deciduous teeth (baby teeth).

When data were available for multiple measures of exposure, the association between health effects and either blood Pb or bone Pb levels was evaluated in this document. While the vast majority of exposure data were in the form of blood Pb measurements, in some cases there was enough data to begin to compare the association between a given health effect for both blood Pb and bone Pb levels. For example, bone Pb in adults appears to be more consistent than blood Pb in its relationship to decreases in specific cognitive measures (specifically in older adults), hypertension, and other cardiovascular effects. Although the relative strength of the association between measures of exposure and the health outcome has not been widely examined, in some cases bone and blood Pb measurements are available in the same group or study and the data have been analyzed in a method that allows such a comparison. For example, multiple studies have reported that blood Pb levels were associated with decreased IQ in the Yugoslavia Prospective Study (see discussion in **Section 4.3.1**). Wasserman *et al.* (2003) demonstrated a stronger association between bone Pb and IQ than for blood in a subset analysis of 167 children with blood and bone Pb measurements. In fact, the association with tibia bone Pb remained significant in a statistical model that controlled for concurrent or average lifetime blood Pb levels.

Pb exposures in the United States have dramatically declined over the last 30 years after bans on Pb in paint, solder, and gasoline, representing a significant public health accomplishment and protection for current populations of children. However, children born in the United States in the 1970s had a mean blood Pb of 15 µg/dL during early childhood. Consequently, health effects in adults today may have been influenced by blood Pb levels >10 µg/dL that many individuals experienced earlier in life.

Keeping these childhood blood Pb levels in mind, there are data on multiple health effects in adults for which studies report a significant relationship with concurrent blood Pb levels (e.g., elevated blood pressure, reduced kidney function, or decreases in specific measures of cognitive function). There is a considerable body of evidence that these health effects are associated with Pb exposure, and multiple studies report a significant association with concurrent blood Pb levels <10 µg/dL. Furthermore the association with blood Pb is supported by the consistency of effects across epidemiological studies, as well as biological coherence with animal data. It is well recognized that the role of early-life Pb exposure cannot be discriminated from the role of concurrent blood Pb without additional long-term studies.

As described in **Section 2.2**, the NTP's conclusions were derived by evaluating data from epidemiological studies with a focus on blood Pb levels <10 µg/dL. The evidence discussed for specific health outcomes within each chapter varies by study design and type of analyses used to examine the relationship of the health outcome with blood Pb across the hundreds of studies evaluated. In some cases, studies examined only groups with blood Pb levels <10 µg/dL, <5 µg/dL, or even lower, and the association of the health effect with the blood Pb level is clear. For example, Lanphear *et al.* (2000) reported that higher blood Pb was associated with lower

academic performance in a cross-sectional study of 4,853 children ages 6-16 from the NHANES III data set. When they analyzed only children with blood Pb <10 µg/dL (n=4,681) or <5 µg/dL (n=4,043), the association with blood Pb was still significant ($p<0.001$ for <10 µg/dL and <5 µg/dL). In other cases, studies reported a significant association between blood Pb and an effect in a group whose *mean* blood Pb level <10 µg/dL (e.g., higher blood Pb level was associated with higher blood pressure in a study of 964 adults in the Baltimore Memory Study (Martin *et al.* 2006)). These analyses support an effect of a blood Pb level <10 µg/dL, but they do not exclude the possibility that individuals significantly above or below the mean blood Pb level are driving the effect, or that past exposure levels are driving the effect. Finally, some studies compared effects between two groups with higher and lower blood Pb levels. For example, Naicker *et al.* (2010) compared the effect of a blood Pb level ≥5 µg/dL with a blood Pb level <5 µg/dL on developmental markers of puberty in 13-year-old girls in South Africa (n=682) and found that blood Pb ≥5 µg/dL was significantly associated with delayed breast development, pubic hair development, and age of menarche.

3.2 Biomarkers of Pb Exposure

The large majority of human epidemiological studies that report individual Pb exposure levels measured Pb in blood samples. This chapter discusses U.S. blood Pb levels and trends for age, gender, and race or ethnicity. Bone Pb has been measured in some studies and is considered to more accurately reflect cumulative body burden of Pb because of the longer half-life of Pb in bone than in blood (reviewed in Barbosa *et al.* 2005, Hu *et al.* 2007). Bone and blood Pb are currently the most useful tools for measuring the body burden of Pb, while measures of Pb in urine and hair are less commonly used and are of low utility (see Hu *et al.* 2007 for review).

While whole-blood Pb is the most readily available biomarker for Pb exposure (and is the basis for this evaluation of Pb levels <10 µg/dL), plasma Pb is the portion of blood Pb that is available to cross cell membranes and enter specific tissues of the body (Cavalleri *et al.* 1978). Plasma Pb represents <5% of the whole-blood Pb concentration, but the proportion of whole-blood Pb in plasma Pb can vary widely and can be influenced by bone Pb levels (e.g., Hernandez-Avila *et al.* 1998, and reviewed in Hu *et al.* 1998). Measuring plasma Pb is technically difficult, requires specialized equipment not widely available, and is not typically measured in research or clinical settings (CDC 2010). Variation in whole blood or plasma collection methods and Pb quantification techniques may limit comparability across studies, particularly when a method with a relatively high level of detection is used in a population with lower Pb exposures.

The National Health and Nutrition Examination Surveys (NHANES) include whole-blood Pb measurements on a cross section of the U.S. population. Specific outcomes in subgroups of the study are routinely published and are included in the chapters of this document covering specific health effects. General trends in blood Pb levels from NHANES data are presented in Figures 7.1, 7.2, and 7.3 (from Mahaffey *et al.* (1982), Brody, *et al.* (1994), and the CDC (2005a, 2011b) including the updated tables for (CDC 2009b)).

Blood Pb levels have decreased over the last 30 years for all age groups (see **Figure 3.1**). The declining blood Pb levels follow declines in Pb exposure related to bans on leaded gasoline, paint, and use of solder in food cans and plumbing in the United States (see **Section 3.3 Sources of Pb**). Prior to the 1970's blood Pb levels were not routinely measured for research purposes, but tooth Pb measurements estimate that peak exposure occurred around 1960 and this is supported by Pb levels in lake sediments (Robbins *et al.* 2010).

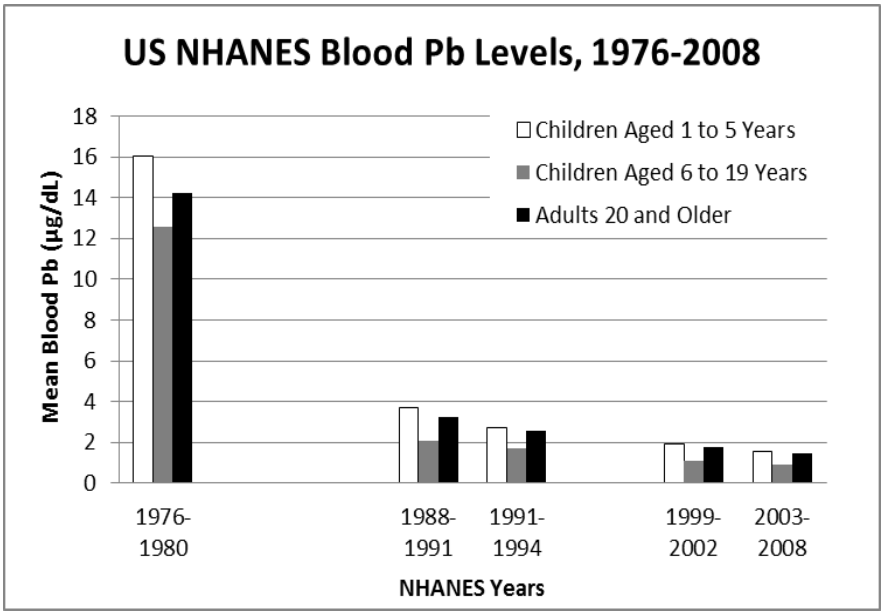

Figure 3.1 U.S. NHANES blood Pb levels for children and adults from 1976-1980 (Mahaffey *et al.* 1982), 1988-1991 (Brody *et al.* 1994), 1991-1994, 1999-2002 (CDC 2005a), and 2003-2008 (CDC (2005a, 2011b)). Years and ages are grouped based on available published data.

Unfortunately, the burden of Pb exposure is not uniformly low in all racial and ethnic subgroups (see **Figure 3.2**). Non-Hispanic blacks have higher blood Pb levels than do non-Hispanic whites across all ages, and being non-Hispanic black is a major risk factor for higher Pb levels in children (Jones *et al.* 2009). When comparing Pb levels for non-Hispanic blacks to those for non-Hispanic whites, almost every age and gender group among blacks had Pb levels statistically significantly higher in both 1991-1994 and 1999-2002 (CDC 2005a). In a

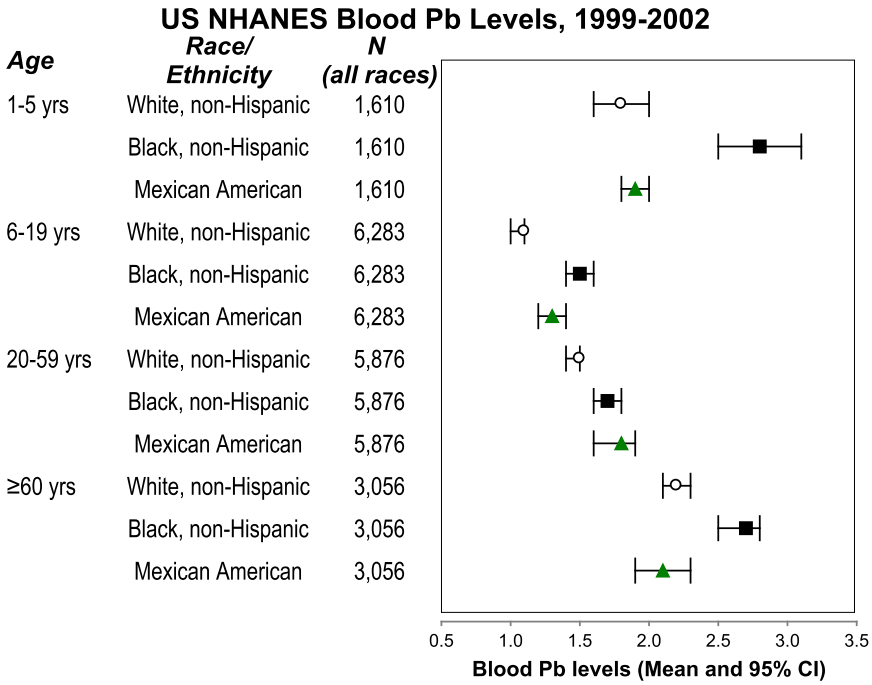

Figure 3.2 U.S. NHANES 1999-2002 mean blood Pb level and 95% CIs for each age category for non-Hispanic whites (open circles), non-Hispanic blacks (filled squares), and Mexican Americans (green triangles) (CDC 2005a)

study of 249 children in Rochester, NY followed from age 6 to 24 months, black children had higher blood Pb levels even after accounting for exposure level and other modifying factors (Lanphear *et al.* 2002). Males also consistently have higher blood Pb levels than do females (see **Figure 3.3**), and this trend was observed in NHANES across most age groups and all racial/ethnic groups (CDC 2005a).

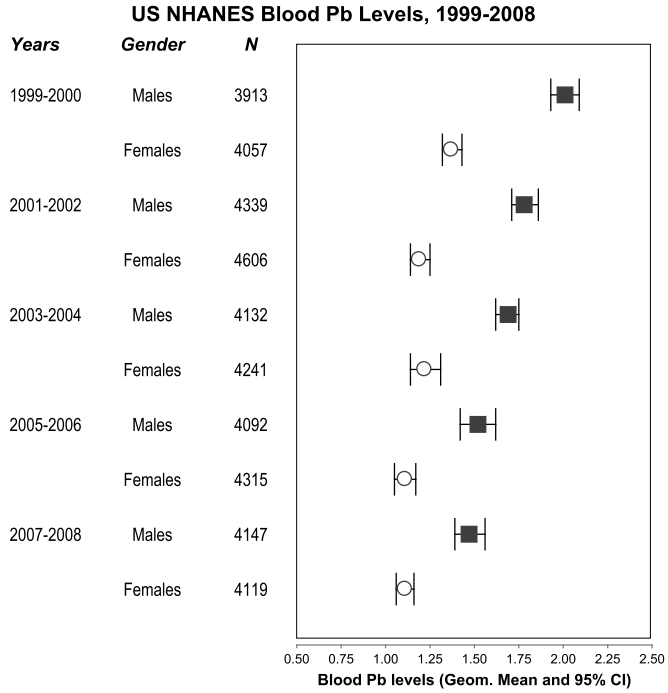

Figure 3.3 U.S. NHANES 1999-2008 mean blood Pb level and 95% CI for all ages for men (squares) and women (circles) (CDC 2011b)

Given an accumulating body burden of Pb and higher past levels of Pb exposure, blood Pb levels are expected to go up with age; however, young children (ages 1-5 years) consistently have higher blood Pb levels than do older children, likely due to hand-to-mouth behavior in this age group (see **Figure 3.2**). Several studies show a peak in children's blood Pb levels around 24 months of age (CDC 2007b). Children are the focus of blood Pb screening and exposure reduction programs because of these higher levels and the established developmental impairments associated with Pb exposure (e.g., CDC's Childhood Lead Poisoning Prevention Program see **http://www.cdc.gov/nceh/lead/about/program.htm**) (CDC 2005b, Clark *et al.* 2011). Blood Pb levels in young children (1-5 years of age) have decreased 10-fold over the last 30 years (geometric means: for 1976-1980, 15.1 µg/dL; for 2007-2008, 1.51 µg/dL (CDC 2007b, 2011b)).

In 2008, only 0.8% of children had confirmed blood Pb levels >10 µg/dL, down from 7.6% in 1997 (**http://www.cdc.gov/nceh/lead/data/national.htm**). However, blood Pb levels have remained consistently higher in non-Hispanic black children, which may be linked to a variety of factors contributing to higher Pb exposure, such as lower socioeconomic status, living in older, urban housing, or having lower calcium intake (see **Figure 3.2**; discussed further in **Section 3.3 Sources of Pb** and **Section 3.4 Modifiers of Pb Exposure**) (Haley and Talbot 2004). Pb exposure in this critical developmental period can have immediate impacts on children's health and contribute to a lifetime of exposure from Pb.

An individual's blood Pb level reflects an equilibrium between current exogenous environmental Pb exposure and the internal (endogenous) body burden of Pb (Factor-Litvak *et al.* 1999, Brown *et al.* 2000, Chuang *et al.* 2001). The body quickly eliminates metals from circulating blood, while bone is a repository for Pb and more accurately reflects the cumulative dose of Pb integrated over years or even decades (reviewed in Hu *et al.* 1998, Barbosa *et al.*

2005, Hu *et al.* 2007). The half-life of Pb in blood is approximately 1 month, while the half-life in bone ranges from 10 to 30 years depending on the bone turnover rate, which varies by type of bone and life stage (Rabinowitz 1991). An estimated 45-70% of blood Pb comes from Pb released from endogenous tissue Pb stores, primarily in bone (Gulson *et al.* 1995). Toxicokinetic models often include other tissues within the model for blood because Pb levels rapidly equilibrate between tissues and blood (e.g., Rabinowitz 1991); however, data on turnover in other organs is limited.

The distribution of Pb in tissues changes with life stage. The distribution is also heterogeneous within bone. Bone and teeth store 90-95% of the total body burden of Pb in adults and from 70% to 95% of the total body burden in children (reviewed in Barbosa *et al.* 2005, Hu *et al.* 2007). Bone Pb was the source of between 40% and 70% of blood Pb in individuals undergoing hip or knee replacement surgery (Smith *et al.* 1996). Pregnancy, lactation, menopause, and osteoporosis are periods of bone demineralization, which may release Pb from bone stores and contribute to increased Pb exposure to other tissues, or to Pb exposure for a developing fetus from Pb released from maternal bone. This hypothesis has been supported by several authors (e.g., Manton *et al.* 2003, Hu *et al.* 2007). In addition, there are data to support higher blood Pb levels in some groups where demineralization is expected; for example, increased blood Pb levels have been demonstrated in postmenopausal women in several studies (e.g., Silbergeld *et al.* 1988, Symanski and Hertz-Picciotto 1995, Webber *et al.* 1995, Korrick *et al.* 2002). In young children, continuous growth results in constant bone remodeling, and bone Pb is likely to be exchanged with blood Pb much more frequently than in adults (reviewed in Barbosa *et al.* 2005, Hu *et al.* 2007). Additional factors that can increase risks to women and children are discussed further in the **Section 3.4 Modifiers of Pb Exposure**.

Bone Pb is typically measured by K-x-ray fluorescence (also called KXRF); however, few research institutions possess this technology and staff trained to use it. The most commonly used KXRF devices have a high detection limit (~10 µg/g bone mineral) and a wide error of measurement, so studies that use this method may underestimate the effect on health. Newer configurations of KXRF with a lower detection limit and less measurement error may improve these estimates, particularly in populations with lower exposure to Pb (Behinaein *et al.* 2011). A more portable x-ray fluorescence device for in vivo (within the body) bone Pb measures was developed using lower energy L-band x-rays which measure Pb concentration in the outermost layer of bone (Nie *et al.* 2011). Both of these methods are impacted by the thickness of skin over the measurement site which may be a concern in health effects related to obesity. Comparison of bone Pb levels between research groups is challenging without a common standard for calibration of instruments. Another modeling approach estimates bone Pb levels from blood Pb measures and covariates typically collected in epidemiological studies (Park *et al.* 2009). This approach could be used to estimate bone Pb in existing studies that do not have the ability to measure bone Pb directly. While blood Pb is by far the most common measure of exposure, it may not be as appropriate as bone Pb, particularly for studies of chronic health conditions (reviewed in Barbosa *et al.* 2005, Hu *et al.* 2007). Physiologically based pharmacokinetic (PBPK) models have been created to combine current blood and bone Pb measures to estimate the Pb

levels at the time of the exposure, allowing a more complete model of the individual's lifetime Pb exposure (Leggett 1993, Coon *et al.* 2006).

Pb has also been measured in hair, urine, and other materials that are easier to obtain; but in general Pb levels fluctuate more rapidly in these materials than in bone. Hair collection is minimally invasive, and hair is easier to ship and store; however, there are no reliable standardized protocols for hair collection, and hair is subject to contamination from environmental sources of Pb (reviewed in Seidel *et al.* 2001, Harkins and Susten 2003, Barbosa *et al.* 2005). In 2001 an ATSDR expert panel concluded that there were too many unresolved scientific issues for hair to be a useful source for evaluating exposures to trace metals, including Pb (ATSDR 2001). Collection of urine is noninvasive, and urine has also been used to measure Pb; however, urine Pb levels vary rapidly and independently of blood Pb and require correction for creatinine levels and glomerular filtration rates to estimate plasma Pb levels at a specific collection time (reviewed in Barbosa *et al.* 2005, Hu *et al.* 2007). Fecal Pb levels reflect both excreted biliary Pb and unabsorbed ingested Pb but must be completely collected over several days to accurately reflect Pb exposures (reviewed in Barbosa *et al.* 2005).

In studies designed to examine reproductive effects, Pb levels in other tissue and fluids have been measured, including semen (e.g., Naha and Manna 2007), ovarian follicles (e.g., Silberstein *et al.* 2006, Al-Saleh *et al.* 2008), and placenta (e.g., Odland *et al.* 2004, Llanos and Ronco 2009, Gundacker *et al.* 2010). Most studies report a single measure of exposure and do not directly compare the relationship between a health effect and different measures of Pb exposure (i.e., tissue Pb compared to blood Pb). Therefore, the usefulness of semen Pb, follicular Pb, or placental Pb as a measure of exposure rather than blood Pb is difficult to ascertain. At this time, blood Pb is a more widely available and is a well-established measure of exposure that is associated with multiple adverse health effects.

Unlike these fluctuating measures, teeth accumulate Pb like other bone, lose Pb at a slower rate than other bone, and for childhood exposure studies, primary teeth (baby teeth) are readily available when lost after 6 years of age (e.g., Manea-Krichten *et al.* 1991). In addition, the layers of the tooth provide a timeline of Pb exposure, including in utero (enamel) and early-childhood (primary tooth dentin) exposures, which may be separately measurable (by laser ablation/inductively coupled plasma/mass spectrometry) without removing the tooth (Uryu *et al.* 2003).

Some studies used indirect measures to estimate Pb exposure, although this is less common because whole-blood Pb measurement has become more widespread. Pb inhibits cytoplasmic enzyme δ-aminolevulinic acid dehydratase (ALAD), which is responsible for heme biosynthesis. Heme is a component of several iron-containing proteins including hemoglobin, the protein that transports oxygen in blood. ALAD can be measured in urine, blood, and plasma and is inversely related to Pb levels (reviewed in Barbosa *et al.* 2005). While not widely used, ALAD levels in blood may be a better marker of long-term exposure than blood Pb measures, but urine ALAD is not sensitive and so is not a good indicator at low Pb exposure levels (Alessio *et al.* 1981, Telisman *et al.* 1982). Pb can also impair heme formation by inhibiting ferrochelatase

such that zinc is used in place of iron, increasing levels of zinc protoporphyrin (ZPP) (reviewed in Barbosa *et al.* 2005). ZPP levels in blood have been used as an indicator of Pb poisoning, but ZPP testing is not sensitive when blood Pb levels are <25 µg/dL (Wildt *et al.* 1987, Parsons *et al.* 1991, Labbe *et al.* 1999).

Pb is cycled through body tissues via several metabolic processes that are influenced by development and life events. Measuring Pb in one tissue at one point in time does not present a complete picture of cumulative Pb exposure, but bone Pb is superior to blood Pb in reflecting the long-term stores of Pb in the body (reviewed in Hu *et al.* 1998, Barbosa *et al.* 2005, Hu *et al.* 2007). However, measuring bone Pb is expensive, requires specialized equipment not widely available, requires study subjects to travel to the location of the K-x-ray fluorescence apparatus, and cannot be done retrospectively on stored samples from large epidemiological studies. In addition, bone Pb levels reflect Pb accumulated over years to decades but measurements of bone Pb do not provide any information regarding the temporal pattern of exposure.

3.3 Sources of Pb

The primary routes of exposure in the general population are oral exposure to Pb from ingesting contaminated water and food or inhaling air and soil containing Pb. For an extensive discussion of environmental sources of Pb, see the EPA's 2006 AQCD (U.S. EPA 2006). Hand-to-mouth behavior in young children increases their risk of exposure to Pb in dust, toys, and paint. Occupational exposures in Pb industries are often associated with elevated Pb levels in workers and can also contribute to Pb exposures in coworkers who do not work with Pb, or in family members exposed to dust brought into the home from the person who works with Pb (Hipkins *et al.* 2004).

Tap water once contributed to as much as 10-20% of total Pb exposure in the United States before amendments to the Clean Water Act (U.S. EPA 2006), and some older pipes, taps, and pre-1986 pipe solder still contain Pb. Source drinking water rarely contains Pb, and the Pb enters tap water through corrosion of Pb from pipes and plumbing fixtures. Corrosion creates exposure from Pb deposits even after previous sources of Pb have been removed from water lines, as well as actual Pb pipes or Pb solder. This corrosion can significantly increase the Pb content in drinking water after changes in water disinfection processes, particularly with use of chloramine (Miranda *et al.* 2007, Jean Brown *et al.* 2011). In a highly publicized incident, the District of Columbia's water supply exceeded the 15 µg/L action level for Pb several times between 2000 and 2004 because of corrosion of Pb scales in service pipes after a switch to chloramine to reduce disinfection byproducts (U.S. EPA 2007). Monitoring in homes with Pb service lines in the district found a small increase in the incidence of blood Pb levels >5 µg/dL, but not over the 10 µg/dL CDC level of concern for children; however, further analysis showed that children in homes with Pb service lines were at risk for blood Pb levels >10 µg/dL even during periods when Pb levels in water were below the action level (CDC 2004, Jean Brown *et al.* 2011). In addition, the incidence of blood Pb levels >10 µg/dL was increased in infants less than 1.3 years of age during the DC drinking water event (Edwards *et al.* 2009). Infants may be at an increased risk from contaminated water if they drink infant formula made with tap water,

because they typically consume 6 oz/kg of formula daily, so infants may have higher exposure relative to body weight than do others in the same household (Bearer 1995).

Dietary Pb sources in the United States have been reduced through several changes in practice, such as removing Pb solder from cans and banning Pb-arsenate pesticides (Bolger *et al.* 1996), and current Pb levels in the U.S. food supply are low (CDC 2010). Contaminated food, particularly if imported from other countries, can be a source of dietary Pb exposure. A study of pregnant women in Monterey, California, identified prepared grasshoppers sent from Oaxaca, Mexico, as a source of Pb poisoning (Handley *et al.* 2007), and tamarind candies imported from Mexico were linked to several cases of Pb poisoning in children (CDC 2002). Spices, herbs, nutritional supplements, and traditional medicines have been shown to contain or be contaminated with Pb as well (Ko 1998, CDC 1999, 2002, Buettner *et al.* 2009, Lin *et al.* 2010). Pottery with a Pb glaze can contaminate food if used for cooking or storage (CDC 2010). While high, acute exposures have been reported from Pb from pottery leaching into food (Matte *et al.* 1994), long-term use may cause a low, chronic exposure and raise the body burden of Pb (Hernandez Avila *et al.* 1991). Use of Pb-glazed ceramics was a major source of cumulative Pb exposure in a study of women in Mexico (Brown *et al.* 2000). Pb crystal glassware can release Pb into alcoholic beverages at levels above the EPA's maximum allowable level for drinking water (Graziano and Blum 1991). In addition, approximately 25% of home-distilled alcohol (moonshine) samples tested by the U.S. Bureau of Alcohol Tobacco and Firearms between 1995 and 2001 had Pb concentrations >400 µg/dL from the use of inappropriate materials in the distillation process (e.g., car radiators or welded metal parts). These concentrations are high enough to produce blood Pb levels >25 µg/dL if one liter was consumed (Morgan *et al.* 2004). Moonshine consumption has been associated with blood Pb levels >15 µg/dL and Pb-related deaths (Pegues *et al.* 1993, Kaufmann *et al.* 2003).

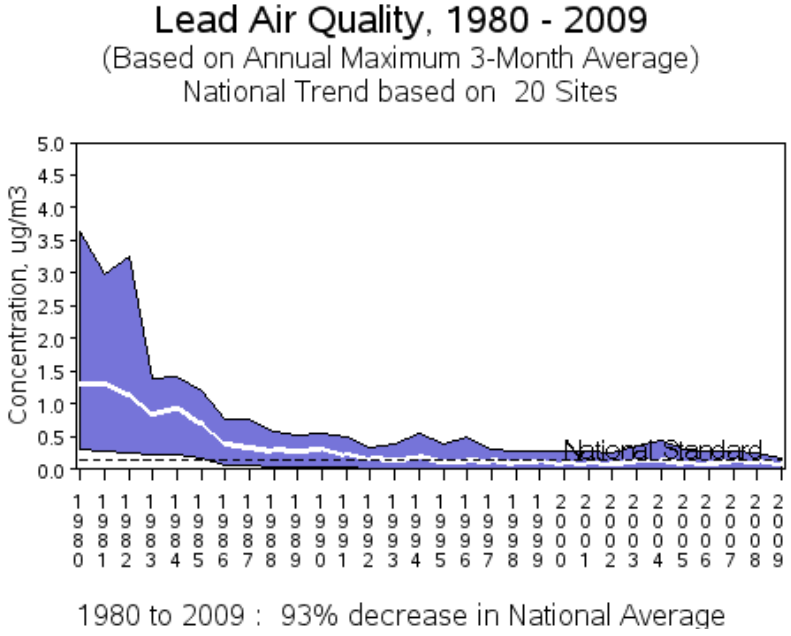

Figure 3.4 U.S. Pb Air Concentration (µg/m^3) from 1980-2009: U.S. Environmental Protection Agency. (http://www.epa.gov/air/airtrends/lead.html, accessed 1 August, 2011)

Inhaled Pb is another source of Pb exposure (U.S. EPA 2006). During the renovation of buildings built before 1978, dust from Pb paint can be inhaled, and residual contamination after a

renovation with inadequate cleanup may continue to expose building occupants to Pb. The U.S. Department of Housing and Urban Development estimated that 40% of U.S. housing contains Pb paint, which presents a potential Pb hazard when it is disturbed or deteriorates (HUD 2001). Leaded gasoline is another inhaled source of Pb in parts of Asia, Eastern Europe, the Middle East, and South America. In the United States, leaded gasoline was banned in 1996 after being phased out for more than 20 years, and average ambient air Pb levels fell 93% between 1980 and 2009 (**Figure 3.4**) (U.S. EPA 2006). While Pb paint and leaded gasoline are no longer major sources of Pb in the United States, Pb from these sources remains in soil and dust, as well as inside people's bodies in bone and other organs as part of the body burden of Pb from earlier exposures to Pb paint and leaded gasoline (U.S. EPA 2006, Zota *et al.* 2011).

Smoking or exposure to passive smoke may lead to increased exposure to Pb in environmental tobacco smoke (ETS). Tobacco itself contains Pb, in part at least, from ambient air sources: the levels of Pb in mainstream smoke from Canadian-grown tobacco cigarettes decreased by 62% from 1968 to 1988 as ambient air Pb levels declined (Rickert and Kaiserman 1994). Serum cotinine (a metabolite of nicotine that can be used as a biomarker of tobacco exposure) and postnatal exposure to ETS were significantly associated with blood Pb levels of children in NHANES III; these levels did not decrease with age, indicating inhalation was more likely than hand-to-mouth behavior in younger children (Lanphear *et al.* 2000, Mannino *et al.* 2003). In studies of outcomes causally linked to ETS exposure, such as neurodevelopment or cardiovascular disease, ETS may confound the observed associations of Pb and the health effect (CDC 2005a).

Contaminated soil also contributes to Pb exposure in humans if inhaled as dust or eaten. Ingested Pb from soil is 26% bioavailable when consumed on an empty stomach and 2.5% bioavailable after a meal (Maddaloni *et al.* 1998). Clay tablets sold in Mexico, Central America, and parts of Africa are eaten for religious reasons, health promotion, or simply taste and texture (CDC 2010). Children and people (particularly pregnant women) with pica, a disorder causing an urge to eat nonfood items, such as dirt or chalk , can also ingest Pb (Klitzman *et al.* 2002).

Children are most commonly exposed to Pb in paint, household dust, and soil—particularly if they reside in pre-1978, deteriorated housing—and can increase their risk of exposure by natural mouthing tendencies (Lanphear *et al.* 1998, U.S. EPA 2006). There are few direct data on Pb absorption from toys or other consumer products, but it is clear that Pb is absorbed from toys in some cases. Pb concentration in toys is mainly associated with use of Pb in paints, coloring agents, and plastic stabilizers in polyvinyl chloride (PVC) plastics (Godoi *et al.* 2009, Greenway and Gerstenberger 2010). A 4-year-old boy had an extremely high Pb level (123 µg/dL blood Pb) after swallowing a vending machine necklace pendant that contained 39% Pb (VanArsdale *et al.* 2004), and similar products could cause lower Pb exposure levels if chewed but not swallowed. Publications have not been identified that provide quantitative measures of the differences in Pb absorption between Pb paint and Pb embedded in plastics as a coloring agent or stabilizer. However, Sanchez-Nazario *et al.* (2003) demonstrated that toy chewing, along with Pb levels in window sills and soil eating habits, were significant predictors

of blood Pb levels in children. Toy chewing may be a route of dust ingestion as well as absorption of Pb from the toy. Children may be exposed to Pb in other consumer products, including plastic window blinds, Pb core candle wicks, or backpacks (Sanborn *et al.* 2002).

Renovating, repairing, or painting a pre-1978 building can release particles of Pb-based paint and is associated with increases in blood Pb levels in children and adults who live in the home (CDC 2009a, 2011a). Thorough cleaning after completion of remodeling is effective in removing most of the Pb dust from a renovated residence (Yiin *et al.* 2004). Proper maintenance of housing by people trained in lead-safe practices, focusing on residential complexes with previous cases of elevated blood Pb levels, can prevent future Pb exposures (CDC 2005b). Construction and painting also contribute to occupational Pb exposures (CDC 2011a). Contractors engaged in renovation or remodeling must be certified through the EPA Lead-Safe Certification Program and use safe work practices to reduce Pb exposures to their clients, employees, and themselves. Additional certification at the state or Federal level is required for abatement to permanently eliminate Pb-based paint hazards from a home (**http://www.epa.gov/lead/pubs/traincert.htm**).

Some hobbies or recreational activities are potential sources of Pb exposure (e.g., Sanborn *et al.* 2002). Hobbies include furniture refinishing, jewelry making, creating stained glass, print-making, enameling copper, casting bronze or lead figurines, leaded glass blowing, working with Pb solder on electronics, and using Pb-containing paints or pottery glazes (CDC 2010). Fishing and hunting can contribute to Pb exposure when making fishing weights, casting ammunition, or eating animals contaminated with Pb after ingesting Pb shot or fishing weights (CDC 2010, 2011a). Air Pb levels in indoor firing ranges were significantly higher in ranges that used powder charges (660 µg/m^3) than in those that used air guns (4.6 µg/m^3) or in archery ranges (0.11 µg/m^3), and blood Pb levels were significantly higher for marksmen using powder charges during the indoor shooting season (Svensson *et al.* 1992). Pb exposures from these hobbies can be significant: a potter and her family experienced elevated Pb levels from Pb glazes used in a home studio (48 µg/dL for the potter, 54 µg/dL for her daughter, and 20 µg/dL for her husband), and a man whose hobbies included melting Pb weights to make figurines and shooting firearms at an indoor firing range had a blood Pb level of 39 µg/dL (Fischbein *et al.* 1992).

Occupational exposures to Pb occur in more than 100 industries where Pb or Pb-containing materials are used or disturbed by workers (CDC 2010). Approximately 95% of all elevated blood Pb levels reported in adults in the United States are work-related (CDC 2011a). The prevalence rate of workers with blood Pb levels >25 µg/dL decreased by more than 50% from 1994 to 2009 (from 14 to 6.3 per 100,000 adult workers), and in 2009 the Adult Blood Lead Epidemiology and Surveillance (ABLES) program lowered their definition for elevated blood Pb level from 25 to 10 µg/dL because of increased concern over health risks from lower blood Pb levels (ABLES 2009). The lowest blood Pb level required to be reported under state laws varies by state; however, of the 10 states that collected all test levels in 2004, 32% of women with blood Pb >5 µg/dL reported occupational exposures, mostly in manufacturing (CDC 2007a). Occupational sources of Pb can also expose workers' families because Pb dust travels home on

clothes and in vehicles (Hipkins *et al.* 2004, CDC 2009c). Living near Pb mining, smelting, and manufacturing sites may expose the surrounding community to low Pb levels, particularly in countries without environmental regulations or monitoring programs (Benin *et al.* 1999). These groups have been the subject of many older studies of health effects associated with Pb exposure and continue to be a source of study subjects with higher exposure levels (e.g., a study of birth outcomes for women living near a Pb smelter plant with a damaged pollution-control device Berkowitz *et al.* 2006).

Because the focus of this evaluation is on blood Pb levels <10 µg/dL, studies with mean blood Pb levels >15 µg/dL were not included in this evaluation except as specified in **Section 8.0 Reproductive / Developmental Effects** (e.g., groups in studies by Kromhout *et al.* (1985) and Locket and Arbuckle (1987) had mean blood Pb levels ≥15 µg/dL and were not included in the evaluation of cardiovascular effects). Stratified analyses of only subjects with Pb levels above and below 10 µg/dL have indicated that associations with some health effects can be stronger at lower exposure levels (e.g., Pb-related intellectual deficits (Lanphear *et al.* 2005)). Excluded occupational studies were mostly older publications on workers with mean blood Pb levels >10 µg/dL or on workers without occupational monitoring programs. Even with the ABLES definition of elevated blood Pb as 10 µg/dL, Pb-exposed workers can have higher blood levels than the general population and a higher lifetime burden of Pb from long-term exposures.

3.4 Modifiers of Pb Exposure

Individual-level differences in exposure and biology affect the amount of Pb that reaches a target tissue to impact health. These differences may influence contact with environmental Pb, as well as Pb metabolism and remobilization of Pb stores. Modifiers of Pb exposure include age, life stage, gender, diet, socioeconomic status, immigrant status, and genetic variants. These factors are often correlated with one another as well.

Blood Pb levels increase with age from bone Pb stores that accumulate over time, as previously discussed in **Section 3.2 Biomarkers of Pb Exposure**. Before bans on Pb in paint, solder, and gasoline, environmental Pb levels in the United States were higher, so older adults accumulated more Pb as children than children do today. Several authors have suggested that the aging process contributes to Pb exposure as bone begins to deteriorate, particularly if coupled with osteoporosis (Silbergeld *et al.* 1988, Campbell and Auinger 2007). The data supporting this hypothesis come from cross-sectional studies and therefore the studies are only able to infer the temporal sequence. A particular challenge comes in relating increased blood Pb levels to mobilization of Pb from bone stores due to osteoporosis, because animal studies have demonstrated that Pb exposure results in lower bone density or bone strength (Hamilton *et al.* 1994, Ronis *et al.* 2001) and support a causal effect of Pb on bone density, rather than the other way around. A study of adults in New York found that age was not a risk factor for higher blood Pb levels (≥10 µg/dL) (Gelberg and Fletcher 2010); however, blood Pb levels <10 µg/dL were not reported. Recent NHANES data support an association between higher blood Pb levels and increased age in older children and adults with generally low blood Pb levels (well below

10 µg/dL; see **Figure 3.2**) (CDC 2005a). Young children are an exception to this age trend and have higher blood Pb levels than do infants and older children (CDC 2007b).

Young children show marked increases in blood Pb levels after birth, with a peak around 2 years of age (Rothenberg *et al.* 1999b). Initially, maternal sources of Pb could contribute to a child's exposure levels. Mothers' blood Pb levels at delivery are highly correlated with umbilical cord blood Pb levels, with umbilical cord blood levels slightly lower (Graziano *et al.* 1990, Rothenberg *et al.* 1999b). The CDC concluded that in utero exposure risks to children are greatest if mothers had a significant past Pb exposure (CDC 2010). Maternal blood and milk Pb levels are correlated as well, but the efficiency of Pb transfer from blood to milk varies at low levels, and Koyashiki *et al.* (2010) concluded that there are no established health risks from breast milk. Current CDC guidelines are to continue breastfeeding up to high blood Pb levels (40 µg/dL blood Pb levels in the mother) (CDC 2010). A study in mice showed that gestational and lactational Pb exposure from the mother increases Pb levels in the offspring, with declining blood Pb levels after weaning (Snyder *et al.* 2000). There is some evidence that Pb from dietary sources is more readily absorbed and retained in young children and infants than in adults (Ziegler *et al.* 1978). Young children are also exposed to environmental Pb because of normal mouthing behaviors, as discussed in **Section 3.3 Sources of Pb**. A number of authors have hypothesized that blood Pb may provide a better measure of Pb exposure in children because of highly active bone remodeling (see reviews by Barbosa *et al.* 2005, Hu *et al.* 2007). However, other than studies that examined Pb levels in shed primary teeth, few studies in children have examined the usefulness of bone Pb data as a measure of exposure in children that might be associated with health effects of Pb.

On average, adult men have higher levels of Pb in blood and bone than do adult women, and men are much more likely to be exposed to occupational sources of Pb. However, women typically go through more stages of life where demineralization of bone may be associated with mobilization of bone stores Pb into circulating Pb. Therefore, a number of authors have supported the hypothesis that women are at risk from increased blood Pb levels mobilized from bone stores during pregnancy and menopause and due to osteoporosis (e.g., Silbergeld *et al.* 1988, Manton *et al.* 2003, Hu *et al.* 2007). Blood Pb levels in pregnant women are generally low in the United States (NHANES geometric mean <5 µg/dL) and do not vary by the age of the mother (Jones *et al.* 2010). Pregnancy-associated increases in blood Pb have been demonstrated in a number of case studies (e.g., Rothenberg *et al.* 1992, Shannon 2003), but the overall pattern of blood Pb throughout pregnancy appears to be complex (Hertz-Picciotto *et al.* 2000, Schell *et al.* 2000). Blood Pb levels follow a U-shaped curve during pregnancy, decreasing during weeks 12-20 and then increasing linearly over the second half of pregnancy (Rothenberg *et al.* 1994). Overall blood Pb levels decrease during subsequent pregnancies, so the first pregnancies pose the most risk of Pb toxicity, particularly if the mother had significant past Pb exposures (Manton *et al.* 2003, CDC 2010). A number of studies have demonstrated increased blood Pb levels in postmenopausal women (e.g., Silbergeld *et al.* 1988, Symanski and Hertz-Picciotto 1995, Webber *et al.* 1995, Korrick *et al.* 2002, Nash *et al.* 2004). Data from Symanski *et al.* (1995) also support a greater relative increase in blood Pb levels in postmenopausal women that have never been pregnant, supporting both increased mobilization of Pb

associated with menopause as well as mobilization and clearing of body burdens of Pb during pregnancy. To a lesser extent, studies also support increased blood Pb levels associated with osteoporosis (e.g., Campbell and Auinger 2007), although as discussed above, studies in laboratory animals demonstrate that Pb exposure causes reduced bone density and therefore cause-and-effect is particularly difficult to establish between osteoporosis and blood Pb levels.

Nutritional deficiencies can be related to Pb levels. Deficiencies in calcium, iron, and zinc were associated with increased Pb levels at 6 months of age, and iron deficiency continued to be associated with Pb at 12 months of age (Schell *et al.* 2004). Low iron intake may contribute by increasing Pb absorption in these infants, who had a mean 12-month blood Pb level of 5.1 µg/dL (Schell *et al.* 2004). In older people, calcium deficiency can increase bone turnover and circulating Pb levels (CDC 2010). Pb absorption is higher when there is less food in the digestive tract, making dietary habits and gastric emptying rates another source of individual variation in the body burden of Pb (James *et al.* 1985, Maddaloni *et al.* 1998).

Low socioeconomic status (SES) is associated with higher blood Pb levels (e.g., Wibowo *et al.* 1986, Greene *et al.* 1992, Schnaas *et al.* 2004, Bellinger 2008). People with low SES may be exposed to a collection of risk factors, including living in older, deteriorated housing with Pb in paint, household dust, pipes, or urban air; consuming diets lower in nutrients and calories; playing with potentially contaminated inexpensive toys; working in jobs with occupational Pb exposure; and other environmental hazards (reviewed in Sexton 1997, Strike and Steptoe 2004). The best strategy for preventing new Pb exposures in housing is to remove the Pb paint and dust, but authors such as Wakefield *et al.* (2002) have noted that Pb abatement can cost over $10,000 per home, and they suggest that this cost may result in remediation of less than 0.1% of seriously dangerous homes per year. Care has to be taken during the remediation, and workers performing the job should receive special training, because as discussed earlier, general repair and renovation can be associated with increased Pb exposure and higher blood Pb levels in building occupants and workers performing the repairs (CDC 2009a, 2011a).

Many immigrants face SES-related exposure risks, but they may have additional risk factors as well. If their home country has relatively high Pb exposure levels, immigrants carry a larger body burden of Pb (CDC 2010). Exposure to leaded gasoline emissions, as estimated from time spent in Mexico City, was a major source of cumulative Pb exposure in a study of postpartum women in Mexico (Brown *et al.* 2000). In a study of pregnant women, a Pb-related increase in blood pressure was only seen in immigrants, predominantly from Latin America, even without markedly higher blood Pb levels than nonimmigrants (Rothenberg *et al.* 1999a). In some cultures, pica during pregnancy is common and accepted (CDC 2010). In a study of pregnant women in New York, pica was the most frequently reported source of Pb exposure (13 women, 39% of those with levels >20 µg/dL) (Klitzman *et al.* 2002). Immigrant status could increase exposure to Pb contaminated products, including alternative remedies, imported cosmetics or food items, or Pb-glazed pottery for cooking or food storage (CDC 2010). Women in the United States using herbal supplements had higher blood Pb levels, particularly in those using St. John's wort or Ayurvedic or traditional Chinese medicinal herbs (Buettner *et al.* 2009).

Biological variation in Pb absorption and metabolism rates can be partially explained by genetic variation. The relationship between Pb exposure and a particular health effect may be modified by the presence of a single nucleotide polymorphism (i.e., variation in a single DNA nucleotide between individuals or groups) or by other genetic variations. When studying genetic risk factors in observational studies, selection for the study is independent of genotype, which remains unknown to the subject, so sources of bias that may confound other risk factors are minimized. If specific genetic variants are found to increase or decrease the association of Pb with a health effect, there is a stronger biological basis for that relationship, and the gene function may give an indication of the mechanism of action. Genes studied for variations in Pb metabolism include hemochromatosis (*HFE*) and aminolevulinate dehydratase (*ALAD*), and specific study details are presented in the appendices for each chapter.

Interactions between many of the previously discussed factors make it difficult to separate the increases in risk from each individual factor. While many measures taken to reduce Pb exposure have decreased blood Pb levels in the U.S. population, economically disadvantaged young children in older housing or pregnant immigrants using contaminated products are still at risk for significant Pb exposures.

3.5 Summary

While Pb can be measured in a variety of human tissues, whole-blood Pb is the most common measure used in both research and clinical settings. Blood Pb levels fluctuate and represent both current exposures from the environment and internal (endogenous) sources of Pb, primarily stored in bone. Bone Pb is a better measure of the cumulative body burden of Pb and therefore it is commonly hypothesized that bone Pb may show more consistent associations with long-term health effects. Pb continues to be used in industrial processes and in manufactured products in the United States and worldwide and is persistent in the environment. Humans are exposed to Pb via water, air, soil, food, and consumer products. Several Pb reduction efforts have significantly reduced exposure levels over the last 30 years, and blood Pb levels have dropped considerably in the United States. Pb exposure levels vary greatly by age, life stage, gender, and socioeconomic level; and even at low levels with blood Pb <10 µg/dL there are health risks. The other chapters of this document outline the evidence for specific health effects from blood Pb levels <10 µg/dL. A discussion of Pb exposures in potentially susceptible populations for specific health effects is included in individual chapters.

4.0 NEUROLOGICAL EFFECTS

4.1 Conclusions:

The NTP concludes that there is *sufficient* evidence that blood Pb levels <5 μg/dL are associated with adverse neurological effects in children and *limited* evidence that blood Pb levels <10 μg/dL are associated with adverse neurological effects in adults. A major strength of the evidence for effects of low-level Pb on neurological outcomes is the consistency of results for an adverse effect of blood Pb <10 μg/dL across multiple indices of neurological effects (e.g., cognition, behavior, and sensory function), through multiple groups, with a wide age range from early childhood to older adults, and from studies using substantially different methods and techniques.

Unlike the data set for most other health outcomes, there are a number of prospective studies of neurological effects include measures of prenatal exposure (either maternal blood or umbilical cord blood). These prospective studies provide *limited* evidence that prenatal exposure to blood Pb levels <5 μg/dL are associated with decreases in measures of general and specific cognitive function evaluated in children. There is also *limited* evidence that prenatal exposure to blood Pb levels <10 μg/dL are associated with decreased IQ, increased incidence of attention-related and antisocial behavior problems, and decreased hearing measured in children. Conclusions on effects of prenatal exposure for outcomes evaluated as children are complicated by the high degree of correlation in childhood blood Pb levels over time, and as described below, blood Pb levels during childhood are also associated with these outcomes.

In children, there is *sufficient* evidence that blood Pb levels <5 μg/dL are associated with broad-based and specific indices of reduced cognitive function and an increase in attention-related behavior diagnosis and antisocial problem behaviors. The association between blood Pb and decreased IQ has been demonstrated in multiple prospective studies of children with blood Pb levels <10 μg/dL, the pooled analyses that reported effects with peak blood Pb levels <7.5 μg/dL (Lanphear *et al.* 2005), and multiple cross-sectional studies that reported effects with mean blood Pb levels <5 μg/dL. Lower levels of academic achievement, as determined by class rank and achievement tests, have been reported in multiple prospective and cross-sectional studies of children with blood Pb <5 μg/dL. An association between blood Pb levels <5 μg/dL and decreases in specific measures of cognitive function have been demonstrated in prospective and cross-sectional studies using a wide range of tests for assessment. Increases in attention-related and problem behaviors are consistently reported in studies with mean blood Pb levels <5 μg/dL. There is *sufficient* evidence that blood Pb levels <10 μg/dL in children are associated with decreased auditory acuity. Multiple cross-sectional studies reported hearing loss, as indicated by higher hearing thresholds and increased latency of BAEPs, in children with blood Pb levels <10 μg/dL.

In adults, there is *limited* evidence that blood Pb levels <10 μg/dL are associated with psychiatric outcomes (including anxiety and depression), decreased auditory function, decreases in specific measures of cognitive function in older adults, and the neurodegenerative

disease ALS. There is *sufficient* evidence that blood Pb levels <10 µg/dL are associated with essential tremor in adults, and *limited* evidence for blood Pb levels <5 µg/dL. As with other studies of health effects of Pb in adults, long-term prospective studies in a group for which blood Pb levels remained consistently below 10 µg/dL from birth until measurement of the outcome of interest would eliminate the potential role of early-life blood Pb levels >10 µg/dL on health effects observed in adults with concurrent blood Pb levels <10 µg/dL. There are more consistent associations between bone Pb and decreases in cognitive function in older adults than for blood Pb, suggesting a role for cumulative Pb exposure.

4.2 How Conclusions Were Reached

Conclusions in the NTP evaluation of Pb-related neurological effects in humans associated with low-level Pb are derived by evaluating the data from epidemiological studies with a focus on blood Pb levels <10 µg/dL. Data reflecting exposure levels up to 15 µg/dL were also considered so that effects at and around 10 µg/dL were not excluded from the evaluation.

There is a relatively large database of human studies for a wide range of neurological effects (see **Table 4.1**) of low-level Pb and therefore, the document makes limited use of the data from laboratory animals to support the human evidence. Major endpoints considered as potential indicators of neurological effects of Pb are listed and briefly described in **Section 4.2.1**. This document is not a review of neurotoxicity, and the reader is directed to published reviews for additional background. Key data and principal studies considered in developing the

Table 4.1: Major neurological effects considered

Effect	Description
Cognitive Function:	
Intelligence quotient (IQ)	**Full-scale IQ** (**FSIQ**), verbal IQ (**VIQ**), and performance IQ (**PIQ**) are evaluated with a variety of tests, e.g., the Wechsler Intelligence Scales for Children (WISC) or the Stanford-Binet Intelligence Scale. WISC is a normative measure for assessing intelligence in children, allowing cross-study comparison (e.g., Lanphear *et al.* 2005).
Academic achievement	Academic performance is measured with a variety of tests, e.g., the Boston Teacher Questionnaire (BTQ), Wide Range Achievement Test-Revised (WRAT-R), and, more recently, end-of-grade testing and standard assessment tests.
General and specific cognitive abilities	Cognitive function is measured with numerous tests including general measures, e.g., the Mental Development Index (MDI) and the General Cognitive Index (GCI); or specific measures such as individual subsets of the WISC, e.g., Block Design or Digit Span.
Behavior and Psychiatric Outcomes	
Behavior	Numerous measures of behavioral outcome are evaluated with tests such as Behavioral Assessment System for Children. **Attention-related behavior** outcomes, including attention deficit hyperactivity disorder (ADHD), are measured with a variety of evaluation tools, e.g., Conners' ADHD/*Diagnostic and Statistical Manual of Mental Disorders* (*DSM*). **Conduct or problem behavior** outcomes are measured with a variety of evaluation tools, e.g., Teacher Report Form-Delinquent Behavior or self-reported delinquent behavior.
Psychiatric outcomes	Mood disorders are diagnosed with various tests, e.g., the Child Behavior Checklist (CBCL) or Brief Symptom Inventory.
Neurodegeneration	
Various diseases	Diagnoses of amyotrophic lateral sclerosis (ALS), Alzheimer's disease, essential tremor, or Parkinson's disease.
Sensory Function	
Audio	Several measures of auditory function, e.g., higher hearing thresholds and altered brainstem auditory evoked potentials (BAEPs).
Vision	Several measures of visual function, e.g., altered visual evoked potentials (VEPs) and electroretinographic (ERG) testing.

NTP conclusions are discussed in detail in **Section 4.3 Evidence for Pb-related Effects on Neurological Outcomes**. The discussion of each neurological effect begins with a statement of the NTP's conclusion that the specific effect is associated with a blood Pb level <10 µg/dL or <5 µg/dL and the age group in which it is identified (childhood or adulthood), as well as the timing of exposure associated with the effect (prenatal, childhood, or concurrent), when available. Although the information necessary to support the NTP's conclusions is presented in **Section 4.3**, the complete data set of human studies considered for evaluation of neurological effects with low-level Pb is included in Appendix A: Neurological Effects, where individual studies are abstracted for further reference. The NTP made extensive use of recent government evaluations of health effects of Pb in the current assessment, and the relevant conclusions of the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) are briefly described in **Section 4.2.2** below.

4.2.1 Principal Measures of Neurological Effects

Table 4.1 lists a number of key neurological endpoints evaluated in epidemiological studies on the effects of Pb exposure and identifies representative tests that have been used to evaluate major neurological effects of Pb. A list or review of the full range of tests and tools used to evaluate neurocognitive, neurobehavioral, psychiatric, and neurophysiological outcomes is beyond the scope of this evaluation. The data available to evaluate each of the major effects are discussed in separate subheadings under **Section 4.3** below.

4.2.2 Principal Conclusions from the 2006 EPA and 2007 ATSDR Pb Documents

The EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) both concluded that negative effects of Pb on neurocognitive ability and neurobehavioral outcomes in children are observed across numerous studies at blood Pb levels <10 µg/dL even after adjusting for confounding factors (see **Table 4.2**). The Lanphear *et al.* (2005) pooled analysis is cited by both EPA and ATSDR as supporting evidence for a decline of up to 6 full-scale IQ (FSIQ) points for an increase in blood Pb from 1 to 10 µg/dL in children. The 2006 EPA AQCD for Lead (U.S. EPA 2006) and the 2005 CDC statement on Preventing Lead Poisoning in Young Children (CDC 2005) highlight the evidence for a supralinear dose-response relationship

Table 4.2: Main conclusions for neurological effects in the 2006 EPA AQCD for Lead and the 2007 ATSDR Toxicological Profile for Lead

"….effects on neurobehavior in children have been observed with remarkable consistency across numerous studies of various designs, populations, and developmental assessment protocols. The negative impacts of Pb on neurocognitive ability and other neurobehavioral outcomes persist in most recent studies even after adjustment for numerous confounding factors, including social class, quality of caregiving, and parental intelligence." (U.S. EPA 2006, pg 6-269)
"…the preponderance of the evidence indicates that lead exposure is associated with decrements in cognitive function. Meta-analyses conducted on cross-sectional studies or a combination of cross-sectional and prospective studies suggest that an IQ decline of 1-5 points is associated with an increase in PbB [blood Pb level] of 10 µg/dL. Most importantly, no threshold for the effects of lead on IQ has been identified…" (ATSDR 2007, pg 25)

for some neurodevelopmental outcomes (particularly IQ) and a steeper dose-response curve at lower Pb levels <10 µg/dL. The EPA 2006 AQCD for Lead (U.S. EPA 2006) also identifies recent evidence that neurocognitive deficits associated with Pb exposure are in turn associated with decreased academic achievement and notes that negative effects of Pb on attention may contribute to underachievement or delinquent behavior in children. The EPA 2006 AQCD for Lead (U.S. EPA 2006) concludes that the negative impacts of Pb on neurocognition and behavior persist into young adulthood and that there is clear evidence that blood Pb levels in children of 5-10 µg/dL (and possibly lower) are associated with these adverse effects. In adults, both ATSDR and EPA noted that chronic occupational Pb exposure is associated with decreased nerve conduction velocity and postural balance abnormalities. The EPA 2006 AQCD for Lead (U.S. EPA 2006) identified ≥14 µg/dL blood Pb level as a possible threshold for these effects, as well as for visuomotor and memory impairment, and effects on the visual and auditory systems (prolonged visual evoked potentials (VEPs) and BAEPs). The EPA 2006 AQCD for Lead (U.S. EPA 2006) characterized the evidence for impairments in cognitive performance associated with Pb levels in adults as mixed, although bone Pb (and therefore long-term cumulative exposure) was associated with decreased cognitive performance. The EPA 2006 AQCD for Lead (U.S. EPA 2006) stated that four studies reported that past occupational exposure to Pb increased the risk of developing ALS and motor neuron disease and two studies reported that essential tremor was associated with low blood Pb levels (a mean of 3 µg/dL). EPA is in the process of revising the AQCD for Lead, and the conclusions of the external draft (U.S. EPA 2012) are largely in line with the 2006 AQCD for Lead (U.S. EPA 2006), plus additional review of the evidence for attention-related behaviors, including ADHD.

The NTP considered the conclusions and data summaries from the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007). In general, the NTP accepted the conclusions and agreed that the data support them. Differences from the ATSDR and EPA documents are identified for specific endpoints.

4.3 Evidence for Pb-related Effects on Neurological Outcomes

4.3.1 Cognitive Function

There is *sufficient* evidence that blood Pb levels <5 µg/dL are associated with decreased cognitive function in children. There are many tests and measures used to evaluate cognitive function and blood Pb levels are associated with decreases in broad-based measures in children such as academic achievement or FSIQ, as well as specific cognitive measures evaluated in all age groups from young children to older adults. No clear and specific pattern of Pb-related decreases in specific cognitive abilities has been identified, although decreased performance on individual domains for attention, executive function, language, learning and memory, and visual-spatial processing have been reported (U.S. EPA 2006, ATSDR 2007). Generally, the lack of clear differences in sensitivity and specificity for individual domains is attributed in part to difficulty discriminating focused effects because test performance covers multiple neurobehavioral processes (U.S. EPA 2006).

The discussion of cognitive function below is divided into three sections: (1) academic achievement—a practical and perhaps more objective measure of cognitive function in children that may relate to achievement in life through education-based achievement measures, (2) IQ in children, and (3) other general and specific measures of cognitive abilities in children and adults.

Academic Achievement

There is *sufficient* evidence that blood Pb levels <5 µg/dL are associated with decreases in various measures of academic achievement in children 6-18 of age (see Appendix A: Neurological Effects for full list of studies). An inverse association between blood Pb level and performance in tests of academic performance, class rank, or end-of-grade testing has been reported in multiple prospective and cross-sectional studies involving children with blood Pb levels from 2 to 10 µg/dL from populations in North America, Europe, and Africa. Studies demonstrated that early-childhood Pb levels in blood (9-36 months age) or tooth dentin (6-8 years of age) are associated with decreased academic achievement measured when children were from 10 to 18 years of age. Data from cross-sectional studies also support a negative effect of concurrent blood Pb levels (between 5 and 10 µg/dL) on academic achievement. However, there is *inadequate* evidence that prenatal blood Pb levels <10 µg/dL are associated with academic achievement in children because of a lack of academic performance studies that include Pb exposure data from prenatal time points.

A number of studies have used academic achievement as an indicator of effects of Pb on cognitive function in children. Many of the earlier studies of academic achievement used tooth dentin Pb as the measure of Pb exposure and compared measures of educational attainment in children with earlier dentin or blood Pb levels. For example, higher tooth dentin Pb levels at 6-8 years of age were associated with: lower class standing in 132 children in Massachusetts at 18 years of age (Needleman *et al.* 1990); reading and spelling difficulties in 8-year-old girls (n=1923 boys and girls) assessed with the Boston Teacher Questionnaire (BTQ) (Leviton *et al.* 1993); and various measures of achievement assessed from 8 to 18 years of age in 1,265 children from the Christchurch Health and Development Study cohort, including number of school certificate passes and Burt Word Reading test assessed at 8, 12, and 18 years of age (Fergusson *et al.* 1988, 1993, 1997). Bellinger *et al.* (1992) reported that blood Pb levels (mean, 6.5 µg/dL) at 2 years of age, but not umbilical cord blood Pb or children's blood Pb levels at 6, 12, 18, or 57 months of age, were significantly associated with lower scores in the Kaufman Test of Educational Achievement (KTEA) in 148 children 10 years of age from the Boston area. In general, the data support a persistent negative effect on cognitive achievement that results in reduced educational attainment. However, at least one study reported that tooth dentin Pb levels were not associated with achievement: Rabinowitz *et al.* (1992) stated that tooth Pb levels were not associated with scores on the BTQ in a study of 493 Taiwanese children.

More recent studies have demonstrated a negative effect of concurrent or early-childhood blood Pb levels on academic achievement at blood Pb levels <10 µg/dL and down to 2 µg/dL. In a cross-sectional study of 4,853 children 6-16 years of age from the NHANES III data set, Lanphear *et al.* (2000) demonstrated that concurrent blood Pb levels <5 µg/dL (geometric

mean, 1.9 µg/dL) were associated with decreased achievement measured by the Wide Range Achievement Test-Revised (WRAT-R). As with other cross-sectional studies of Pb, an important limitation is that blood Pb during early childhood is likely to have been higher than blood Pb measured during the study; and therefore, blood Pb may have been >10 µg/dL at earlier time points for children in this study. In a cross-sectional analysis of 511 U.S. children between 6 and 10 years of age, concurrent blood Pb ≥5 µg/dL was associated with lower scores on the Wechsler Individual Achievement Test (Surkan *et al.* 2007). In a cross-sectional study of 533 girls in Saudi Arabia between 6 and 12 years of age, Al-Saleh *et al.* (2001) reported that class rank percentile was inversely associated with concurrent blood Pb levels (mean, 8 µg/dL). Wang *et al.* (2002) found a similar inverse relationship between concurrent blood Pb level (mean, 5.5 µg/dL) and class ranking of 934 Taiwanese children 9 years of age for individual subject areas (e.g., natural sciences and Chinese). Min *et al.* (2009) reported that blood Pb <5 µg/dL measured at 4 years of age was significantly associated with decreased school reading scores (Woodcock-Johnson III Tests of Achievement) at 9 and 11 years of age in a subgroup analysis as part of a prospective study of 278 inner-city children from Cleveland, OH. Chandramouli *et al.* (2009) demonstrated that educational performance on standard achievement tests at 7-8 years of age was inversely associated with blood Pb levels ≥5 µg/dL measured at 30 months of age in a prospective study of 582 children in the United Kingdom. Surkan *et al.* (2007) reported that blood Pb of 5-10 µg/dL was associated with decreased performance in the Wechsler Individual Achievement Test in a study of 6- to 11-year-old children in Maine and Massachusetts. Miranda *et al.* (2007) reported that blood Pb levels down to 2 µg/dL (collected as part of Pb screening programs when the children were <5 years of age) were negatively related to test performance in both reading and mathematics in a study of 8,603 children in the fourth grade (9- and 10-year-olds) from four counties in North Carolina. In an expanded study of over 57,000 children in the fourth grade from across North Carolina, who were screened for blood Pb between 9 months and 3 years of age, Miranda *et al.* (2009) demonstrated a similar effect of blood Pb levels down to 2 µg/dL on end-of-grade reading scores compared to the reference group with blood Pb levels of 1 µg/dL.

No studies were located that evaluated maternal Pb levels and educational achievement; however, Bellinger *et al.* (1992) reported that umbilical cord blood Pb levels (29% of the study group had levels ≥10 µg/dL) were not significantly associated with lower scores in the KTEA in 148 children 10 years of age from the Boston area.

Confounding variables were considered in all of the studies listed above to some degree, but the studies of Miranda *et al.* (2007, 2009), Chandramouli *et al.* (2009), and Lanphear *et al.* (2000) included a large number of confounders, such as socioeconomic variables, sex, race/ethnicity, age of blood Pb measurement, parental education, and tobacco exposure, and demonstrated that the effects on achievement were independent of known confounders. The Miranda *et al.* (2009) study found evidence of differential effects in students with lower test scores, reporting that children with the lowest test scores had a larger adverse effect of blood Pb on lowering the test score. Lower SES and lower parental education also had a larger adverse effect on children with lower test scores, suggesting that Pb and other confounders all have a greater impact in individuals that already have lower levels of achievement. The studies

of Surkan *et al.* (2007) and Bellinger *et al.* (1992) reported a negative effect of Pb on academic achievement while controlling for the child's IQ, suggesting that IQ and academic performance may serve as somewhat independent measures of cognitive function.

Summary of support for conclusions

Animal data support a Pb-associated decrease in neurobehavioral tests of learning including fixed-interval operant conditioning at blood Pb levels ≥11 μg/dL (see U.S. EPA 2006 for recent reviews of the animal data, ATSDR 2007). The human data include multiple prospective and cross-sectional studies supporting a Pb-associated decrease in educational attainment at blood Pb levels from 2 to 10 μg/dL. The conclusion of *sufficient* evidence for decreased academic achievement in children 6-18 years of age with blood Pb levels <5 μg/dL measured in early childhood through 18 years of age is based on the consistency of effects on several measures of academic achievement in multiple studies. The Bellinger *et al.* (1992) study includes multiple blood Pb measurements and only found a negative effect of blood Pb measured at 2 years of age on later life academic performance. Multiple studies (e.g., Miranda *et al.* 2007, Chandramouli *et al.* 2009, Miranda *et al.* 2009) reported that early-childhood Pb exposure is associated with later performance. However, clear evidence that early-childhood Pb exposure is associated with academic performance at later ages is complicated by the high degree of correlation in childhood blood Pb levels over time (e.g., see Dietrich *et al.* 1993a, Lanphear *et al.* 2005). Current evidence can be used to support the importance of both early-life exposure and current blood Pb levels. The conclusion of *inadequate* evidence that prenatal blood Pb levels <10 μg/dL are associated with academic achievement in children is based on the lack of studies of academic performance in children with Pb exposure data from prenatal time points. The NTP's conclusions for *sufficient* evidence that decreased academic achievement in children 6-18 years of age is associated with Pb levels <5 μg/dL extends the conclusions from EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's 2007 Toxicological Profile for Lead (ATSDR 2007), which were limited to blood Pb levels <10 μg/dL; however, the EPA's 2012 draft (U.S. EPA 2012) currently supports a lower blood Pb level.

IQ

There is *sufficient* evidence that blood Pb levels <5 μg/dL are associated with decreases in FSIQ, a global measure of cognitive ability in children 4-13 years of age (see Appendix A: Neurological Effects for full list of studies). The NTP conclusion of *sufficient* evidence that blood Pb levels <5 μg/dL are associated with decreased IQ is based on several lines of evidence, including multiple cross-sectional studies of children reporting lower IQ associated with mean blood Pb levels <5 μg/dL, pooled analyses demonstrating reduced IQ with peak blood Pb levels <7.5 μg/dL, and multiple prospective studies reporting decreased IQ at blood Pb levels <10 μg/dL. Multiple prospective studies demonstrated that blood Pb levels <10 μg/dL during early childhood are associated with decreased IQ measured in children 6-13 years of age from populations in North America, Australia, Europe, and Asia by a range of different tests to assess IQ. Many recent studies have used the Wechsler Intelligence Scales for Children (WISC) and have adopted similar protocols to assist in cross-study comparison and greater generalization of the findings. The most recently published pooled analysis of IQ data, Lanphear *et al.* (2005), took advantage of the similarity in study design of seven prospective studies and concluded

that blood Pb levels <10 μg/dL in children (and specifically in children with maximal blood Pb levels <7.5 μg/dL) are associated with intellectual deficits. The pooled analysis of Lanphear *et al.* (2005) estimated that 3.9 IQ points (95% CI: 2.4, 5.3) were lost with an increase in blood Pb from 2.4 μg/dL to 10 μg/dL. Data from cross-sectional studies also support an association between concurrent blood Pb levels <5 μg/dL and decreased IQ in children up to 13 years of age. The evidence is mixed for an association between maternal or umbilical cord blood Pb and decreased IQ in children evaluated at a later age. Therefore, there is *limited* evidence that prenatal blood Pb levels <10 μg/dL are associated with decreased IQ in children. There is some evidence that the association between decreased IQ might be more consistent for bone Pb than for blood Pb, but those data come from a group known to have blood Pb levels >10 μg/dL during early childhood.

A number of prospective studies have reported an association between blood Pb <10 μg/dL and decreased IQ score in children from 4 to 13 years of age. The decrease in IQ has been reported in groups for whom the blood Pb level remained <10 μg/dL from birth to evaluation. For example, in a prospective study of 148 children with serial blood Pb measurements from birth through 10 years of age, Bellinger *et al.* (1992) (reanalyzed in Bellinger and Needleman 2003) demonstrated that blood Pb levels at 2 years of age (mean, 6.5 μg/dL), but not other ages, were significantly associated with decreases in FSIQ and verbal IQ (VIQ) assessed by the WISC-R at 10 years of age in a cohort from the Brigham and Women's Hospital; the association with performance IQ (PIQ) was not significant (p=0.091). Min *et al.* (2009) demonstrated that blood Pb measured at 4 years of age (mean, 7 μg/dL) was significantly associated with decreased FSIQ by the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-R) at 4 years of age and with decreased FSIQ by the full WISC-IV at 9 and 11 years of age in a prospective study of 278 inner-city children from Cleveland, OH. However, many of the early studies were in children that had blood Pb levels >10 μg/dL at some stage before they were given a test to evaluate IQ. For example, Baghurst *et al.* (1992) reported that blood Pb levels during early childhood up to 4 years of age (but not maternal blood Pb, umbilical cord blood Pb, or average through 7 years of age) were significantly associated with decreased FSIQ and VIQ scores evaluated by the WISC-R at 7 years of age in 494 children in Port Pirie, Australia; however, mean blood Pb levels in this cohort were >10 μg/dL after birth. A similar association between blood Pb and decreased FSIQ has been demonstrated in a number of prospective studies with cumulative mean blood Pb levels >10 μg/dL that evaluated IQ in children at 5-13 years of age (Dietrich *et al.* 1993b, Tong *et al.* 1996, Wasserman *et al.* 1997, Factor-Litvak *et al.* 1999, Canfield *et al.* 2003a, Wasserman *et al.* 2003, Jusko *et al.* 2008).

An association between concurrent blood Pb <10 μg/dL as well as <5 μg/dL and decreased IQ has been reported in multiple studies. For example, the two studies by Chiodo *et al.* (2004, 2007) evaluated a range of neurodevelopmental endpoints in 240 and 500 African-American inner-city children 7-9 years of age with mean concurrent blood Pb levels of 5.4 μg/dL; these levels were related to decreased FSIQ, PIQ, and VIQ, as well as behavioral endpoints evaluated with the WISC-III. When grouped by cutoff points of 10, 7.5, 5, and 3 μg/dL blood Pb, the study supported significant effects on IQ at blood Pb levels of ≥5 μg/dL, with some support for effects at ≥3 μg/dL. In a cross-sectional study of 261 children 8-11 years of age in Korea, Kim *et al.*

(2009) reported that concurrent blood Pb (mean, 1.7 µg/dL) was significantly associated with reduced FSIQ and VIQ as measured by the Korean Educational Development Institute—Wechsler Intelligence Scales for Children test. This study represents the cohort with the lowest mean blood Pb level associated with decreased IQ; however, because the Kim *et al.* (2009) was cross-sectional in nature, and did not follow the children from birth, it does not provide information to verify that blood Pb levels were <5 or <10 µg/dL from birth to the age at which the test was administered.

In a study of 253 children from the Cincinnati Lead Study, Dietrich *et al.* (1993b) reported that decreases in FSIQ and PIQ by WISC-R were significantly associated with concurrent blood Pb levels at 6 years of age and blood Pb for the year before (down to age 3 for PIQ); however, IQ was not associated with maternal blood Pb, umbilical cord blood Pb, or blood Pb levels during early childhood. Hornung *et al.* (2009) analyzed the age of greatest susceptibility for blood Pb to contribute to lower IQ scores as evaluated with the WISC-R in a combined cohort from the Cincinnati and Rochester lead studies (n=397 total), with peak blood Pb mean of 13.6 µg/dL and concurrent blood Pb at 6 years of age = 6 µg/dL. At 6 years of age, blood Pb measured concurrently (β=-3.48; $p<0.001$) and the prior year (at age 5; β=-4.39; $p<0.001$) had the strongest effect on IQ, compared to blood Pb at earlier ages (e.g., age 1; β=-0.08;$p=0.934$). Note that peak childhood blood Pb in the Cincinnati and Rochester cohorts was >10 µg/dL; therefore, it is not clear that the observed decreases in IQ reported in Dietrich *et al.* (1993b) and Hornung *et al.* (2009) are strictly associated with a blood Pb level <10 µg/dL.

No clear evidence was located that maternal or umbilical cord blood Pb levels <10 µg/dL are associated with decreased IQ in children. Many studies, such as Baghurst *et al.* (1992), Bellinger *et al.* (1992), and Dietrich *et al.* (1993b) described earlier, did not find a significant association with prenatal blood Pb levels in groups for which prenatal blood Pb was <10 µg/dL. In a prospective study that examined the relationship between maternal blood Pb during early and late pregnancy and FSIQ in children as measured by the WISC-R, Schnaas *et al.* (2006) reported that maternal blood Pb levels at 28-36 weeks of pregnancy (mean, 7.8 µg/dL) were related to reduced IQ in children 6-10 years of age. However, as in other studies, the mean blood Pb in this cohort was >10 µg/dL during early childhood.

The evidence for a Pb-related decrease in IQ has been the subject of four key meta-analyses combining data across several studies (Needleman and Gatsonis 1990, Pocock *et al.* 1994, Schwartz 1994, Lanphear *et al.* 2005) and was extensively reviewed in the 2006 EPA AQCD for Lead (U.S. EPA 2006) and ATSDR Toxicological Profile for Lead (ATSDR 2007). These analyses provide strong support that blood Pb and tooth Pb are significantly associated with decreases in IQ in children; however, many of the cross-sectional and prospective studies included in these analyses are based on cohorts with blood Pb levels >10 µg/dL at some age between birth and age of IQ test. The Schwartz *et al.* (1994) and Lanphear *et al.* (2005) meta-analyses support effects at blood Pb levels <10 µg/dL (and with peak blood Pb levels <7.5 µg/dL), although these analyses face challenges of confounding and the different tests used to assess IQ across studies. However, the 2005 pooled analysis (Lanphear *et al.* 2005) reduced these confounders by pooling data from seven prospective studies that used similar test protocols (e.g., relying

principally on the WISC) and included 1,333 children from several countries. The analyses supported an association between blood Pb levels <10 µg/dL and decreased IQ, and the authors concluded that blood Pb levels <10 µg/dL (and specifically <7.5 µg/dL) in children are associated with intellectual deficits. Lanphear *et al.* (2005) reported significant decreases in IQ in analyses restricted to children with a maximum blood Pb level <10 µg/dL. The authors also attempted to characterize the shape of the dose-response between blood Pb and IQ (addressed further in the following discussion).

Although some earlier studies of Pb and cognitive function did not include adjustment for maternal IQ or other confounders, these studies were also generally in children with blood Pb levels >10 µg/dL. More recent studies of the relationship between blood Pb and IQ in children with blood Pb levels <10 µg/dL considered a wide range of potential confounders. For example, the Lanphear *et al.* (2005) pooled analysis included maternal IQ, maternal education, score for the Home Observation for Measurement of the Environment (an assessment of the stimulation in the environment in which the child is raised), and birth weight in the final model; however, prenatal smoking, prenatal alcohol use, mother's marital status, maternal age, the child's sex, and birth order were also considered and did not to influence the analyses. Some studies have also considered the effects of co-exposure to other metals or toxicants. For example, Kim *et al.* (2009) demonstrated that children with higher levels of manganese in their blood had greater decreases in IQ for a given level of Pb; the results of this study suggest that co-exposure to other metals should be considered in studies of cognitive effects of Pb.

In one example, the extensive data on potential covariates and the thorough characterization of exposure measurements based on serial blood Pb assessments over time allowed comparison of the strength of the association between Pb and decreased IQ for two measures of exposure: blood Pb and bone Pb. As part of the Yugoslavia Prospective Study, 290 children were assessed with the WISC-III at 10-12 years of age, and 167 of these individuals had with tibia bone Pb measurements (Wasserman *et al.* 2003). Both bone Pb and average lifetime blood Pb levels were significantly associated with decreased FSIQ, PIQ, and VIQ. Bone and blood Pb measurements were highly correlated (concurrent Pb r=0.75; lifetime average Pb r=0.85; p<0.01) in children from Titova Mitrovica (a Pb smelter town with higher Pb exposure levels) but not in Pristina, the town with lower Pb exposure levels. Tibia Pb was a significant predictor of FSIQ or PIQ with or without adjustment for current or average lifetime blood Pb levels. In contrast, average or current blood Pb levels were not associated with IQ in models that included tibia Pb levels. Therefore, the authors concluded that the association with decreased IQ is stronger for bone Pb than for blood Pb (Wasserman *et al.* 2003). In is important to note two points in this analysis. First, blood Pb measurements were >10 µg/dL for most children in these groups. Second, this is one of the few studies in children that examined the usefulness of bone Pb data as a measure of exposure, although a number of studies have measured Pb levels in shed deciduous teeth.

Shape of the dose-response curve

There is abundant discussion in the Pb literature on the shape of the dose-response curve in the lower range of exposure (i.e., at blood Pb levels <10 µg/dL) for neurodevelopmental effects of

Pb. This discussion centers around several studies that have reported greater neurocognitive effects (principally on IQ and specific measures of cognitive function) of an incremental increase in blood Pb levels at lower concentrations compared to the effects for an incremental increase at higher blood Pb levels (Canfield *et al.* 2003a, Lanphear *et al.* 2005, Rothenberg and Rothenberg 2005, Kordas *et al.* 2006). This indicates a steeper slope (greater effect per unit increase) in the dose-response curve at lower blood Pb levels. The 2006 EPA AQCD for Lead (U.S. EPA 2006) discusses this issue extensively and reviews the evidence that the dose-response curve has a steeper slope at lower blood Pb levels. The 2005 CDC (CDC 2005) review of the epidemiological evidence for neurological effects in children also noted that there was evidence for a steeper slope in the dose-response curve at lower blood Pb levels. Evaluation of the shape of the dose-response curve is beyond the scope of the current evaluation.

Summary of support for conclusions

Animal data support a Pb-associated decrease in neurobehavioral tests of learning (including fixed interval operant conditioning) at blood Pb levels ≥11 µg/dL (see U.S. EPA 2006 for recent reviews of the animal data, ATSDR 2007). The human data include several meta-analyses and prospective and cross-sectional studies that support an association between blood Pb levels <10 µg/dL and lower FSIQ scores in children 4-13 years of age. The conclusion of *sufficient* evidence that decreases in IQ scores in children are associated with blood Pb levels <10 µg/dL measured in early childhood or in concurrent blood Pb samples is based on consistent evidence for decreased IQ across multiple studies and in well-accepted pooled analyses (e.g., Lanphear *et al.* 2005). Multiple studies (e.g., Baghurst *et al.* 1992, Bellinger *et al.* 1992, Min *et al.* 2009) reported that early-childhood (2-4 years of age) Pb exposure is associated with IQ score in children at later ages. Clear evidence that early-childhood exposure is associated with decreased IQ at later ages is complicated by the high degree of correlation in childhood blood Pb levels over time (e.g., see Dietrich *et al.* 1993a, Lanphear *et al.* 2005). The conclusion of *limited* evidence that prenatal blood Pb levels <10 µg/dL are associated with decreased IQ in children is based on mixed evidence for an association with maternal or umbilical cord blood Pb. The NTP's conclusions for *sufficient* evidence that blood Pb levels <10 µg/dL are associated with decreased IQ in children 4-13 years of age is consistent with the conclusions from EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's 2007 Toxicological Profile for Lead (ATSDR 2007).

Other general and specific measures of cognitive function

There is *sufficient* evidence that blood Pb levels <5 µg/dL are associated with decreases in various general and specific measures of cognitive function in children from 3 months to 16 years of age (see Appendix A: Neurological Effects for full list of studies). An association between increased blood Pb level and decreases in specific cognitive abilities has been demonstrated in prospective and cross-sectional studies using a wide range of tests to assess cognitive function. Although Pb-related decreases have been reported in multiple cognitive measures, including individual domains for attention, executive function, language, learning and memory, and visual-spatial processing, the lack of a clear pattern of effects has contributed to difficulty in distinguishing specific, focused effects, because test performance covers multiple neurobehavioral processes (U.S. EPA 2006, ATSDR 2007). Although relatively few studies have

examined the potential effects of Pb in adults without occupational exposure, a number of studies have reported Pb-associated decreases in specific measures of cognitive function in groups of older adults, such as the Normative Aging Study. Several of these studies reported an association between concurrent blood Pb levels <10 µg/dL and decreased performance in the Mini-Mental State Examination (MMSE) and other cognitive measures. Six studies also demonstrated that bone Pb levels were associated with decreased cognitive performance. However, other studies did not find an association with concurrent blood Pb levels. There is *limited* evidence that blood Pb levels <10 µg/dL are associated with decreases in specific measures of cognitive function in older adults, because of the mixed results for an association with blood Pb and the more consistent support for an association with bone Pb.

There are a large number of tests used to evaluate cognitive function, with tests specific for subjects of different ages and designed to explore different cognitive domains (see Appendix A: Neurological Effects under outcomes measured for tests used in individual studies). Two of the more common tests that demonstrated effects of Pb in younger subjects measure general cognitive function: the Mental Developmental Index (MDI) from the Bayley Scales of Infant Development and the overall General Cognitive Index (GCI) from the McCarthy Scales of Children's Abilities and individual measures. Data on older children evaluated with the WISC were discussed earlier in the context of Pb-related decreases of IQ, which is also a general measure of cognitive function; however, the WISC also includes more specific subsets, such as Block Design and Digit Span, and multiple studies reported decreases in scores on WISC subsets at blood Pb levels <5 µg/dL (lower than the 10 µg/dL blood Pb levels generally associated with effects on FSIQ). Multiple tests are used to evaluate cognitive function in adults, including the MMSE.

An association between increased blood Pb level and decreased MDI score was demonstrated in multiple studies at blood Pb levels from <2 to 10 µg/dL. Maternal blood Pb levels <10 µg/dL and umbilical cord blood Pb levels <5 µg/dL have been reported to be associated with decreased cognitive performance in children up to 3 years of age by the MDI. Recent studies (Gomaa *et al.* 2002, Al-Saleh *et al.* 2009, Jedrychowski *et al.* 2009a, Jedrychowski *et al.* 2009b, Pilsner *et al.* 2010) reported effects in children with blood Pb levels that remained consistently below 10 µg/dL; however, early-childhood blood Pb increased to levels >10 µg/dL for some of the groups examined. Two recent studies reported that concurrent blood Pb levels <10 µg/dL are associated with decreased MDI scores, but there is less evidence that concurrent blood Pb is associated with MDI than there is for an association with measures of prenatal Pb exposure.

The overwhelming majority of prospective studies found that prenatal exposure determined by either umbilical cord or maternal blood Pb levels <10 µg/dL were associated with decreased MDI in children through 3 years of age. Significantly lower MDI scores in children tested at 3-36 months of age were associated with prenatal exposure based on umbilical cord blood Pb at the following levels: ≥1 µg/dL in children evaluated at 24 and 36 months of age in a study of 457 children in Poland (effects significant for boys and girls combined, but likely driven by boys `(Jedrychowski *et al.* 2009a, Jedrychowski *et al.* 2009b)); 2.7 µg/dL with MDI evaluated in 6-month-old children in Saudi Arabia (n=119) (Al-Saleh *et al.* 2009); 6.4 µg/dL with MDI evaluated

in 3-month-old children from the Cincinnati Lead Study (n=266) (Dietrich *et al.* 1987); 6.6 µg/dL with MDI evaluated in children 6, 12, 18, and 24 months of age from Brigham and Women's Hospital (n=182-249) (Bellinger *et al.* 1984, Bellinger *et al.* 1986, Bellinger *et al.* 1987); 6.7 µg/dL with MDI evaluated in 24-month-old children from Mexico City (n=197) (Gomaa *et al.* 2002, Pilsner *et al.* 2010); and 9.2 µg/dL evaluated at 3, 6, and 12 months of age in a study of 133 children in China (Shen *et al.* 1998). Although most of the studies demonstrated a Pb-associated decrease in MDI, Cooney *et al.* (1989a) reported that umbilical cord and maternal blood Pb levels (mean, 8-9 µg/dL) in participants in the Sydney Lead Study (n=215-274) were not related to change in MDI tested at 6, 12, 24, or 36 months of age.

Several prospective studies reported an association between maternal blood Pb and decreased MDI, with no relationship to umbilical cord blood or a less consistent relationship (e.g., Dietrich *et al.* 1987, Ernhart *et al.* 1987, 1988, 1990, Hu *et al.* 2006). Hu *et al.* (2006) examined maternal blood Pb during the first, second, and third trimester of pregnancy and found that first-trimester blood Pb level (mean, 7 µg/dL) was associated with lower MDI in children at 24 months of age in a study of 146 mother-infant pairs from Mexico City; however, the association with umbilical cord blood Pb or maternal Pb during other time periods was not significant. Dietrich *et al.* (1987, 1990) reported that maternal blood Pb sampled during pregnancy (mean, 8 µg/dL) was associated with decreased MDI tested at 3, 6, and 24 months of age in children from the Cincinnati Lead Study and that umbilical cord blood was only associated with MDI evaluated at 3 months of age. Ernhart *et al.* (1987) reported that decreased MDI evaluated at 6 months of age was associated with maternal blood Pb at delivery (mean, 6.5 µg/dL) in children from the Cleveland Lead Study; however, neither umbilical cord blood Pb or maternal Pb was associated with MDI evaluated at later time points (1-3 years of age). It is important to note that the blood Pb of infants from both the Cincinnati and Cleveland lead studies increased after birth and that childhood blood Pb levels for these groups were >10 µg/dL (mean, 16-17 µg/dL at 2 years of age), therefore, the lack of association with prenatal Pb levels may be influenced by the high childhood blood Pb levels. A follow-up study by Ernhart *et al.* (1988) did not find an association with MDI and early-childhood blood Pb or concurrent blood Pb levels; however, as noted above, the blood Pb levels in this cohort was >10 µg/dL. In contrast, Bellinger *et al.* (1990) found that postnatal blood Pb was associated with a change in cognitive performance from 24 months of age (evaluated by the MDI) to 57 months of age (evaluated by the GCI) in a study of a group with a similar age range.

Few studies have examined the relationship between MDI and measures of exposure other than blood Pb. Gomma *et al.* (2002) reported that maternal patellar (knee cap) Pb level was significantly associated with a decrease in MDI scores evaluated in children at 24 months of age in a study of 197 mother-infant pairs in Mexico City.

Several studies have also demonstrated that concurrent blood Pb levels <10 µg/dL were associated with lower MDI scores in children from 6 to 36 months of age. For example, Solon *et al.* (2008) reported that concurrent blood Pb (mean, 7.1 µg/dL) was associated with decreased MDI in children from 6 to 36 months of age. Similar results (Pb-related decrease in MDI) were

reported in children evaluated at 24 months of age in Mexico City (Tellez-Rojo *et al.* 2006) with mean concurrent blood Pb level of 4.9 µg/dL.

Similar to the data supporting a negative effect of blood Pb on MDI scores, findings from several studies support an association with decreased performance on the GCI in children. In a study of 170 children from the Brigham and Women's Hospital, Bellinger *et al.* (1991) reported that GCI scores and the McCarthy subscale score for perceptual performance evaluated at 57 months of age were inversely associated with blood Pb levels in the children at 24 months (mean, 6.4 µg/dL) but not at other ages. Similarly, Schnaas *et al.* (2000) reported that blood Pb levels from 24 to 36 months of age were associated with decreased performance on the GCI, but effects at earlier ages or later ages up to 56 months were not significant, in a study of 112 children from the Mexico City Prospective Study. Blood Pb was consistently below 10 µg/dL in the cohorts from both the Bellinger *et al.* (1991) and Schnaas *et al.* (2000) studies. It is also important to note that some studies did not find a significant association between blood Pb and performance on the GCI; for example, maternal blood Pb (9 µg/dL), umbilical cord Pb (8 µg/dL), or current blood Pb were not associated with performance on the GCI in a study of 207 children from the Sydney Lead Study evaluated at 48 months of age (Cooney *et al.* 1989b).

Multiples studies have reported that concurrent and early-childhood blood Pb levels <10 µg/dL are associated with decreases in specific indices of cognitive function in children from 4 to 16 years of age. Examples include studies demonstrating decreased performance on subsets of the WISC. In a cross-sectional study of 384 children in Germany, Walkowiak *et al.* (1998) reported that concurrent blood Pb (mean, 4.7 µg/dL) in 6-year-olds was associated with decreased vocabulary scores evaluated as part of the German version of the WISC. In a large cross-sectional study of children 6-16 years of age from the NHANES III data set, Lanphear *et al.* (2000) and Krieg *et al.* (2010) demonstrated that concurrent blood Pb levels <10 µg/dL (geometric mean, 1.9 µg/dL) were associated with decrements in the Block Design and Digit Span subsets of the WISC-R. In subgroup analysis, Min *et al.* (2009) reported that blood Pb <5 µg/dL measured at 4 years of age was significantly associated with decreased performance by the WPPSI-R at 4 years of age and with perceptual reasoning scores of the WISC at 9 years of age in a prospective study of 278 inner-city children from Cleveland, OH. Chiodo *et al.* (2004, 2007) evaluated a range of neurocognitive effects in inner-city African American children 7-9 years of age (n=243 and 506, respectively) with concurrent blood Pb levels <10 µg/dL (mean 5.4 µg/dL; blood Pb levels were related to decreased performance in multiple tests, including the Block Design and Digit Span subsets of the WISC and various tests of executive function, memory, and attention. When grouped by cutoff points of 10, 7.5, 5, and 3 µg/dL blood Pb, the studies found significant effects on some tests at levels of ≥3 µg/dL (e.g., Block Design).

Most studies of the effects of Pb on cognitive function in adults involved occupationally exposed individuals with blood Pb levels >10 µg/dL, and fewer studies have been reported in adults from the general population. A number of studies in older adults reported a decrease in cognitive function associated with Pb, with more consistent evidence for an association with bone Pb than for concurrent blood Pb levels. Payton *et al.* (1998) reported that concurrent blood Pb (mean, 5.5 µg/dL) levels in 141 older men (mean age, 67 years) from the Normative

Aging Study were associated with decreases in specific measures of cognitive function from a battery of tests administered, including slower pattern comparison speed, vocabulary, word list memory, constructional praxis, and the Boston Naming Test. Tibia Pb level (but not patellar Pb) was associated with decreased performance in a test of spatial ability. In a study of 736 older men (mean age, 69 years) also from the Normative Aging Study, Wright *et al.* (2003) reported that blood Pb (mean, 4.5 μg/dL), patellar Pb, and tibia Pb were all associated with decreased performance on the MMSE. Muldoon *et al.* (1996) evaluated cognitive performance with the MMSE and Wechsler Adult Intelligence Scale-Revised for 530 older women (mean age, 71 years) either from rural residents in Pennsylvania or from urban dwellers in Baltimore. Blood Pb (mean, 4.8 μg/dL) was associated with decreased performance on the Trail Making Test (for blood Pb >8 μg/dL, OR=2.60 (95% CI: 1.04, 6.49); for blood Pb 4-7 μg/dL, OR=2.05 (95% CI: 1.05, 4.02); relative to referents with blood Pb ≤3 μg/dL) and the Digit Symbol Substitution test (for blood Pb >8 μg/dL, OR=3.73 (95% CI: 1.57, 8.84); for blood Pb 4-7 μg/dL, OR=2.03 (95% CI: 1.06, 3.88); relative to referents with blood Pb ≤3 μg/dL), but only in the rural population and not the urban population (Muldoon *et al.* 1996).

There are also several studies that reported an association between bone Pb and decreased performance but did not find an association with blood Pb levels. Shih *et al.* (2006) reported a lack of an association between current blood Pb (mean, 3.5 μg/dL) and cognitive function in a study of 985 older adults in the Baltimore Memory Study (mean age, 60 years). However, tibia Pb levels were significantly associated with lower scores in all seven domains of the cognitive test battery (Shih *et al.* 2006). Weuve *et al.* (2009), reported that tibia Pb levels were associated with reduced cognitive function by the Telephone Interview for Cognitive Status in a study of 587 older women (mean age, 61 years) from the Nurses Health Study; blood Pb and patellar Pb levels were not significantly related to the test score. Two studies did not find an association with blood Pb levels and did not collect bone Pb data: Nordberg *et al.* (2000) did not find an association between blood Pb level (mean, 3.7 μg/dL) and performance on the MMSE in a study of 762 older adults (mean age, 88 years) in Sweden; Gao *et al.* (2008) reported that concurrent blood Pb (mean, 3.9 μg/dL) was not significantly related with cognitive function in as study of 188 people (mean age, 69 years) from rural China assessed with a test battery that included the Community Screening Interview for Dementia.

Several studies have reported a greater effect on changes in cognitive function over time, rather than a single cross-sectional examination. Weisskopf *et al.* (2004) tested cognitive function in 466 men (mean age, 67 years) from the Normative Aging Study over several years and reported that higher patella Pb was associated with a greater decline in performance on the MMSE over a 3.5-year period between retesting; they found no association with blood Pb (mean, 4 μg/dL), and the association with tibia Pb level was weaker. In an expanded study of the same group covering 1,089 men, Weisskopf *et al.* (2007) reported similar results, stating that there was little association between blood or bone Pb levels and cognitive test scores on a cross-sectional basis; however, patellar and tibia Pb levels were associated with decline in performance on a range of cognitive functions, particularly visuospatial and visuomotor subscales. Bandeen-Roche (2009) reported that tibia Pb was associated with decreased hand-eye coordination over time in a study of 964 older adults from the Baltimore Memory Study (59

years of age at baseline), but not with other measures of cognitive function in a battery of 20 standardized tests.

Fewer studies have examined cognitive performance in younger adults with low blood Pb levels. In a series of studies from Krieg *et al.* in 4,937 adults 20-59 years of age from the NHANES III data set, blood Pb levels <10 µg/dL in adults were not associated with performance on neurobehavioral tests. Krieg *et al.* (2005, 2009, 2009, 2010) did not find a significant relationship between blood Pb (mean, 3.3 µg/dL) and neurobehavioral tests for simple reaction time, symbol-digit substitution, and serial digit learning. In a portion of the study that included both children and adults from the same NHANES data set, Krieg *et al.* (2010) demonstrated that vitamin D receptor (*VDR*) genotype did affect the relationship between blood Pb and performance on the WISC-R Digit Span and WRAT-R math scores; however, they found no clear pattern in terms of the effect of *VDR* genotype on the relationship between blood Pb and cognitive function.

Most of the recent studies of the relationship between blood Pb and specific measures of cognitive function considered a range of potential confounders, such as age, sex, education, and race/ethnicity. Some studies have also examined potential physical, genetic, and psychological confounders. In a study of 47 health adults 55-67 years of age in Rochester, NY, van Wijngaarden *et al.* (2009) found that higher tibia and calcaneus (heel bone) Pb levels were significantly correlated with measures of memory impairment; however, the relationship with bone Pb was not significant after adjusting for hypertension. Several studies of cognitive function in children and adults have also investigated the potential modifying effect of gene polymorphisms (e.g., *ALAD*, *HFE*, *APOE*, and *VDR* genotypes) and other factors (Weuve *et al.* 2006, Rajan *et al.* 2008, Glass *et al.* 2009, Krieg *et al.* 2010). In particular, Wang *et al.* (2007) demonstrated a significant effect of *HFE* polymorphism on the rate of decline in MMSE score in 358 participants from the Normative Aging Study. Krieg *et al.* (2010) and Rajan *et al.* (2008) reported that there was no clear pattern of *ALAD* or *VDR* genes modifying the relationship of Pb and cognitive function. Glass *et al.* (2009) found that tibia Pb was associated with impaired executive function and that there was a significant interaction with neighborhood psychosocial hazards in a study of 1,001 older adults (mean age, 59 years) from the Baltimore Memory Study. Surkan *et al.* (2008) reported that higher maternal self-esteem attenuated the Pb-associated decrease in MDI score in a study of 309 children 2 years of age from Mexico City. Peters *et al.* (2010) reported that blood Pb (mean, 5 µg/dL) was significantly associated with decreased cognition as measured by the MMSE, but that bone Pb and stress were modifiers of the association with Pb in a study of 811 older men (mean age, 68 years) in Normative Aging Study.

Summary of support for conclusions

Animal data support a Pb-associated decrease in neurobehavioral tests of learning (including fixed interval operant conditioning) at blood Pb levels ≥11 µg/dL and mixed evidence for effects on memory (see U.S. EPA 2006 for recent reviews of the animal data, ATSDR 2007). The human data include multiple prospective and cross-sectional studies supporting a Pb-associated decrease in specific measures of cognitive function in children at blood Pb levels from 1 to

10 µg/dL. The conclusion of *sufficient* evidence for decreases in specific measures of cognitive function in children 3 months to 16 years of age with blood Pb levels <5 µg/dL measured in concurrent blood or in early childhood is based on the consistency of effects on multiple measures of cognitive function in multiple studies. Multiple studies (e.g., Min *et al.* 2009) reported that early-childhood Pb exposure is associated with decreases in cognitive function observed at later ages. However, as discussed for IQ, clear evidence that early-childhood or prenatal Pb exposure is associated with decreases in specific indices of cognitive function at later ages is complicated by the high degree of correlation in childhood blood Pb levels over time (e.g., see Dietrich *et al.* 1993a, Lanphear *et al.* 2005). Multiple studies (e.g., Bellinger *et al.* 1984, Al-Saleh *et al.* 2009, Jedrychowski *et al.* 2009a) also reported that levels of Pb in umbilical cord blood are associated with decreases in cognitive function observed at later ages by cognitive tests such as the MDI. The conclusion of *limited* evidence that prenatal blood Pb levels <5 µg/dL are associated decreases in specific measures of cognitive function in children is based on strong consistent support for an association between umbilical cord blood and MDI scores, and the mixed evidence for maternal blood Pb and MDI or other measures of cognitive function in children. The conclusion of *limited* evidence that blood Pb levels <10 µg/dL are associated with decreases in specific measures of cognitive function in older adults is based on the mixed evidence that concurrent blood Pb levels <10 µg/dL are associated with reduced cognitive function and the consistent support that bone Pb is associated with decreases in specific measures of cognitive function or with change in these measures through time in older adults. The EPA's 2006 AQCD for Lead (U.S. EPA 2006) suggests that cumulative exposure to Pb may be critical in contributing to neurocognitive deficits in adults because of the significant associations with bone Pb; EPA also highlights the mixed evidence for an association with blood Pb. As with other studies of health effects of Pb in adults, prospective studies in a group for which the data demonstrated that blood Pb levels remained consistently below 10 µg/dL from birth until measurement of the outcome of interest would eliminate the potential role of early-life blood Pb levels above 10 µg/dL on health effects observed in adults with concurrent blood Pb levels <10 µg/dL. The NTP's conclusion of *sufficient* evidence that blood Pb levels <5 µg/dL are associated with decreases in general and specific measures of cognitive function in children 3 months to 16 years of age extends the conclusions from EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's 2007 Toxicological Profile for Lead (ATSDR 2007), which were limited to blood Pb levels <10 µg/dL; however, the EPA's 2012 draft (U.S. EPA 2012) currently supports a lower blood Pb level.

4.3.2 Behavior

Attention-related Behaviors

There is *sufficient* evidence that blood Pb levels <5 µg/dL are associated with attention-related behavioral problems in children 3-18 years of age (see Appendix A: Neurological Effects for full list of studies). This conclusion is for an association with attention-related behaviors, rather than ADHD alone, for two reasons: (1) "attention-related behaviors" is an inclusive expression that more accurately reflects the data supporting a range of Pb-associated behavioral effects in the area of attention, of which ADHD is one example on the more severe end of the spectrum of effects; and (2) in the available studies, designations of "ADHD" in children generally lack the

strength of an ADHD diagnosis by trained clinicians using *DSM-IV-TR* criteria. Most of the studies that demonstrated a significant association between Pb and attention-related behaviors are studies that report behavioral testing and concurrent blood Pb levels. Increased diagnosis of ADHD and attention-related behaviors (e.g., inattention and hyperactivity) are consistently reported in studies with mean blood Pb levels <5 μg/dL and in several studies at blood Pb levels <2 μg/dL. The diagnostic criteria for attention-related behaviors can include multiple categories of behavioral deficits in addition to inattention, hyperactivity, and the overall diagnosis of ADHD (Aguiar *et al.* 2010). A review of individual behavioral domains , such as response inhibition, flexibility, and planning aspects, was published by Eubig *et al.* (2010); however, data on those individual domains will not be covered here because a sufficient number of studies examine overall ADHD diagnosis, inattention, and hyperactivity to develop more general conclusions on the overall classification of attention-related behaviors. There is *limited* evidence that prenatal blood Pb levels <10 μg/dL are associated with attention-related behaviors in childhood. Several prospective studies reported that Pb exposure determined from maternal blood during pregnancy, umbilical cord blood, or early-childhood blood (up to 30 months) or from tooth dentin levels (6-8 years of age) is associated with inattention or hyperactivity as children or young adults[2] (7-20 years of age); however, early-childhood blood Pb levels in some of these individuals were >10 μg/dL; therefore, the effect may not be strictly associated with blood Pb levels <10 μg/dL.

A clear majority of recent cross-sectional studies (more than 10 publications since 2000) demonstrate an association between current blood Pb at mean levels from 1 to 11 μg/dL and attention-related behavioral problems, ADHD, or other indicators of decreased attention or increased hypersensitivity in children from 3 to 18 years of age. Multiple studies reported an association with blood Pb of ≤5 μg/dL. In a case-control study of 1,260 children 4-12 years of age in China (n=630 ADHD cases and 630 matched controls), Wang *et al.* (2008) reported a significant association between concurrent blood Pb levels >5 μg/dL and ADHD determined from a structured diagnostic interview. An increase in attention-related behavioral problems, particularly inattention, was observed on the teachers' and parents' ratings using a German questionnaire version of the Conners' Rating Scale (namely the Fremdbeurteilungsbogenfür Auf- merksamkeits-Hyperaktivitäts-Störung (FBB-ADHS)) and German-KITAP (Testbatterie zur Aufmerksamkeitsprüfung fü Kinder) test battery in a study of 83 children 8-12 years of age in Romania with concurrent mean blood Pb levels 3-5 μg/dL (Nicolescu *et al.* 2010). Two studies by Chiodo *et al.* (2004, 2007) evaluated a range of neurodevelopmental measures in inner-city African American children 7-9 years of age (n=243 and 506, respectively) with concurrent blood Pb levels (mean, 5.4 μg/dL); blood Pb levels were related to higher ADHD and inattention scores on the Barkley-DuPaul Scale, greater hyperactivity on the PROBS-14 Problem Behavior Scale, and poor attention on the Achenbach Child Behavior Checklist Teacher Report Form. When they grouped the children by cutoff points of 10, 7.5, 5, and 3 μg/dL blood Pb, the two studies supported significant effects on inattention at blood Pb levels of ≥3 μg/dL.

[2] Note: children are defined as <18 years of age in this document; therefore, 18- to 20-year-olds in the study are considered adults.

Two publications that evaluated ADHD in children from the NHANES (1999-2002) data set (Braun *et al.* 2006, Froehlich *et al.* 2009) reported an association between concurrent blood Pb levels of 1-2 μg/dL and ADHD. Braun *et al.* (2006) reported a significant increase in the odds ratio ((OR=4.1 (95% CI: 1.2, 14.0; p=0.001)) for ADHD among children 4-15 years of age with blood Pb levels >2 μg/dL compared to referents with blood Pb 0.8 μg/dL (n=4,704 participants); the determination of ADHD was based on parental reported previous diagnosis or use of stimulant medication. In children 8-15 years of age from the same NHANES data set (n=2,588), Froehlich *et al.* (2009) found that children with concurrent blood Pb >1.3 μg/dL (the third tertile of Pb exposure) had a significantly greater odds ratio (OR=2.3 (95% CI: 1.5, 3.8; p=0.001)) and children with blood Pb ≥0.9 to 1.3 μg/dL had an OR=1.7 (95% CI: 0.97, 2.9; p=0.06) of ADHD compared to referents (the first tertile of Pb exposure) with 0.8 μg/dL blood Pb; the determination of ADHD was based the Diagnostic Interview Schedule for Children.

Several other cross-sectional studies support an association between concurrent blood Pb levels of ≤2 μg/dL and attention-related behaviors, ADHD, or symptoms of inattention and or hyperactivity. In a cross-sectional study of 639 children 8-11 years of age in Korea, Cho *et al.* (2010) reported a significant association between blood Pb levels (mean, 1.9 μg/dL) and ADHD ratings in the inattention, hyperactivity, and total scores using the teacher evaluation of the Korean version of the ADHD rating scale. Higher concurrent blood Pb level (mean, 1.8 μg/dL) was associated with increased score on the Korean version of the abbreviated Conners' scale for ADHD in a study of 1,778 children 7 years of age in South Korea in the Children's Health and Environment Research Study (Ha *et al.* 2009). In a pair of studies of 236 children, Nigg *et al.* (2008, 2010) reported that concurrent blood Pb levels were significantly higher in ADHD-combined type children 6-17 years of age than in controls, and that blood Pb levels (mean, 1 μg/dL) were significantly correlated with aspects of ADHD diagnosis by two experienced clinicians, including hyperactivity, oppositional behaviors, ADHD index, and attention problems evaluated with the Conners' ADHD Rating Scale, revised. Nigg *et al.* (2008, 2010) found that measures of hyperactivity-impulsivity were more consistently associated with blood Pb measurements than were inattention symptoms.

However, the consistent association with hyperactivity over inattention observed by Nigg *et al.* (2008, 2010) is not universal, and several other studies have found an association with blood Pb and ADHD or measures of inattention. Kim *et al.* (2010) found increased inattention and hyperactivity symptoms on the teacher-completed Korean version of the ADHD Rating Scale in children with blood Pb ≥2.2 μg/dL compared to referents with lower blood Pb levels in a study of 275 South Korean children 8-10 years of age. Roy *et al.* (2009) reported an increase in the ADHD index (β=0.17; p=0.05), and ratings on the *DSM-IV* inattentive scale (β=0.24; p=0.01), but not ratings on the *DSM-IV* hyperactive scale (β=0.17; p=0.13) by the Connors' ADHD /*Diagnostic and Statistical Manual of Mental Disorders* (4th edition (*DMS-IV*)) scales, in 3- to 7-year-old children in India with concurrent mean blood Pb of 11 μg/dL; increased anxiety and social problems were also noted. Inattention and hyperactivity in 11-year-olds evaluated by the Rutter Parent and Teacher Behavior Questionnaires were also correlated with blood Pb (mean, 11 μg/dL) in a cross-sectional study of 579 children from the Dunedin Multidisciplinary Health and Development Study in New Zealand (Silva *et al.* 1988). Canfield *et al.* (2003b) reported that

blood Pb at 4 years of age was associated with decreased focused attention by the Shape School Task at 4-5 years of age in 172 children in Rochester, NY.

The relationship between concurrent blood Pb levels and attention-related behaviors is supported by multiple studies, but there are also data supporting a role for early-life prenatal or early-childhood Pb exposure and attention-related behaviors in older children. In a prospective study with Pb exposure measures spanning prenatal and early childhood to 6 years of age, Ris *et al.* (2004) reported that attention in 15- to 17-year-old children by the Continuous Performance Test – Conners' Version was inversely associated with maternal blood Pb during the first or second trimester of pregnancy (mean, 8.9 µg/dL), average childhood blood Pb <5 years of age, and blood Pb at 6.5 years of age in a study of 195 children from the Cincinnati Lead Study); however, blood Pb levels during early childhood were above 10 µg/dL in this cohort (Dietrich *et al.* 1993a, Wright *et al.* 2008), so it is not clear that this is strictly associated with blood Pb levels <10 µg/dL. Chandramouli *et al.* (2009) found that attention in 7- and 8-year-olds in the United Kingdom, as assessed with the Test of Everyday Attention for Children, was not significantly related to blood Pb (mean, 4.2 µg/dL) at 30 months of age (n=582), but there was a greater odds for teacher-rated hyperactivity (OR=2.82 (95% CI: 1.08, 7.35)) in children with blood Pb >10 µg/dL. Cord blood Pb (mean, 6.8 µg/dL) and tooth dentin Pb were associated with inflexible behavior in 8-year-olds in the Task domain of the BTQ, but there was no relationship with hyperactivity, in their study of 1,923 children in Boston (Leviton *et al.* 1993). Fergusson (1993) reported a significant association between tooth dentin Pb of shed primary teeth (6-8 years of age) and measures of inattention and restlessness at 12-13 years of age by the Rutter and Conners parental and teacher questionnaires in a study of 1,265 children from the Christchurch Health and Development Study cohort. In a similar study of 79 young adults[3] 19-20 years of age, Bellinger *et al.* (1994a) reported that both dentin Pb levels in shed teeth (6-8 years of age) and tibia Pb levels were significantly associated with specific measures of attention.

Summary of support for conclusions

Animal data support a Pb-associated reduced performance on neurobehavioral tasks (including increased distractibility), with effects observed down to approximately 10 µg/dL (e.g., deficits in waiting behavior in rats Brockel and Cory-Slechta 1998, U.S. EPA 2006 for recent reviews of the animal data, see ATSDR 2007). In general the animal data are unlikely to represent thresholds because the lowest levels of effect have yet to be studied. The human data supporting a Pb-associated increase in attention-related behaviors such as ADHD, inattention, and hyperactivity include multiple cross-sectional studies of children from 3 to 18 years of age with blood Pb levels of 1-11 µg/dL. The conclusion of *sufficient* evidence for a positive association with attention-related behaviors in children at blood Pb levels <5 µg/dL is based on the consistency of effects in these studies and supports effects down to and below 2 µg/dL blood Pb. This conclusion is for an association with attention-related behaviors rather than ADHD alone for two reasons. First, "attention-related behaviors" is a more inclusive term that more accurately

[3] Note: children are defined as <18 years of age in this document; therefore, 19- and 20-year-olds in the study are considered adults.

reflects the support for a range of Pb-associated behavioral changes in the area of attention, of which ADHD is one example on the more severe end of the spectrum of effects. Second, diagnostic criteria in the available studies, with the exception of the two Nigg *et al.* (2008, 2010) reports, are based largely on the parent reporting a physician's diagnosis, untrained teacher evaluations, or that the child is taking ADHD medication; therefore, they lack the additional strength that would be provided by studies that incorporate diagnostic evaluations by trained clinicians or physicians to identify ADHD using *DSM-IV-TR* criteria. Recent studies found effects on attention-related behaviors after controlling for a large number of confounders, such as socioeconomic variables, sex, race/ethnicity, age of blood Pb measurement, parental education, and tobacco exposure. Several studies reported that blood Pb levels were significantly associated with ADHD even after controlling for potential mediating effects of child IQ (e.g., Nigg *et al.* 2008, Nigg *et al.* 2010). Although several studies reported an association between concurrent blood Pb and attention-related behaviors in children up to 15 or 17 years of age (Braun *et al.* 2006, Nigg *et al.* 2008, Froehlich *et al.* 2009, Nigg *et al.* 2010), or between bone Pb measured as children and attention evaluated as young adults 19 and 20 years of age (Bellinger *et al.* 1994a), no studies were located that examined the relationship between blood Pb levels in adults and ADHD or attention-related behaviors. There is *inadequate* evidence to evaluate the potential association between blood Pb <10 µg/dL and effects on attention-related behaviors in adults. The conclusion of *limited* evidence for an association between blood Pb levels and attention-related behaviors in children with prenatal Pb exposure at blood Pb levels <10 µg/dL is based on the evidence for an association with prenatal blood Pb <10 µg/dL that may include childhood exposure >10 µg/dL, with support from the concurrent blood Pb data and a number of bone Pb studies reporting an association with attention-related behaviors such as inattention or hyperactivity. Existing data support the importance of current Pb exposure for attention-related behaviors but does not allow a clear distinction between the role of early-life Pb exposure and current exposure. The NTP's conclusions for *sufficient* evidence that attention-related behaviors in children 3-18 years of age are associated with Pb levels <5 µg/dL are stronger than the limited relationship with behavioral features of ADHD outlined in the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's 2007 Toxicological Profile for Lead (ATSDR 2007); however, given the growth of the database in recent years, the EPA's 2012 draft (U.S. EPA 2012) currently supports an association with ADHD and biological plausibility supported by Pb-associated increases in attention-related behaviors.

Problem Behaviors

The discussion of problem behaviors below is divided into two sections: (1) delinquent, criminal, or antisocial behavior; and (2) psychiatric outcomes. Most of the studies of Pb effects on problem behaviors focused on studies of conduct problems or criminal behavior. Recent studies of mood disorders or psychiatric outcomes such as anxiety and depression have also reported an association with blood Pb levels.

Delinquent, Criminal, or Antisocial Behaviors

There is *sufficient* evidence that blood Pb levels <5 µg/dL are associated with antisocial behavioral problems or actual criminal behavior in children from 6 to 15 years of age (see Appendix A: Neurological Effects for full list of studies). Recent studies, including studies with

large sample sizes such as the NHANES data sets, have reported effects down to blood Pb levels <1 μg/dL, and a number of cross-sectional studies demonstrated an association between concurrent blood Pb at and below 10 μg/dL and antisocial problem behaviors. Multiple studies reported that bone Pb and tooth dentin Pb are related to antisocial behavior problems. There is *limited* evidence that prenatal blood Pb levels <10 μg/dL are associated behavioral problems in childhood. A number of prospective studies have reported a significant association between prenatal blood Pb <10 μg/dL and delinquent behavior or criminal arrests as children; however, childhood blood Pb levels in some of these individuals are >10 μg/dL; therefore, the effect may not be strictly associated with blood Pb levels <10 μg/dL. Several studies have also reported that umbilical cord blood Pb was not associated with antisocial problem behaviors in children. Although several studies have reported an association between prenatal Pb levels <10 μg/dL and criminal arrests in young adults[4] up to 24 years of age, no studies of antisocial behavioral problems were located with Pb levels in adults. There is *inadequate* evidence to evaluate the potential association between blood Pb <10 μg/dL and effects on behavior problems in adults.

Braun *et al.* (2008) reported a significant increase in the odds ratio (OR=7.24 (95% CI: 1.1, 49.5)) for conduct disorder among children 8-15 years of age with blood Pb levels ≥0.8 μg/dL compared to referents with blood Pb ≤0.7 μg/dL (conduct disorder measured using criteria described in *DSM-IV*; n= 3,081 from the NHANES 2001-2004 data set). This study extended the findings from earlier cross-sectional studies that found associations between current blood Pb at and above 10 μg/dL and behavioral problems or criminal behavior in children from 6 to 13 years of age (i.e., mean blood Pb 10-15μg/dL in Yule *et al.* 1984, Silva *et al.* 1988, Thomson *et al.* 1989, Burns *et al.* 1999). For example, in a cross-sectional study of 11-year-old children (n=579) in New Zealand, blood Pb (mean, 11 μg/dL) was significantly correlated with behavioral problems evaluated with the Rutter Parent and Teacher Behavior Questionnaires (Silva *et al.* 1988). Burns *et al.* (1999) reported that increased behavioral problem scores by the Child Behavior Checklist (CBCL) were associated with lifetime average blood Pb (geometric mean, 14 μg/dL) in a study of 322 children 11-13 years of age from the birth cohort in Port Pirie, Australia.

Several prospective studies support an effect of Pb exposure on antisocial problem behaviors using Pb exposure determined by blood Pb or bone Pb (Bellinger *et al.* 1994b, Dietrich *et al.* 2001, Wasserman *et al.* 2001, Chen *et al.* 2007). Results of some studies support a stronger or more consistent relationship between bone Pb or concurrent blood Pb and behavioral outcomes than for prenatal or early-childhood blood Pb. For example, Bellinger *et al.* (1994b) reported that problem behaviors in 8-year-old children in Boston (n=1,782 judged by the Teacher Report Form of the Child Behavior Profile) were significantly associated with tooth dentin Pb levels; however, there was no association with umbilical cord blood Pb levels (mean, 7 μg/dL). Behavioral measures in a study of 7-year-old children (n=780) demonstrated an association with concurrent blood Pb (mean, 8 μg/dL) for both indirect effects on behavior symptoms mediated through IQ and direct effects on school problems and behavioral

[4] Note: children are defined as <18 years of age in this document; therefore, 19- to 24-year-olds in these studies are considered adults.

symptoms index evaluated with the Behavioral Assessment System for Children (Chen *et al.* 2007); however, there was no association with earlier blood Pb levels that were significantly above 10 μg/dL at 2 years of age (mean, 26 μg/dL) or 5 years of age (mean, 12 μg/dL).

In contrast, other prospective studies support an association with prenatal or early-childhood exposure with problem behaviors at a later age. Dietrich *et al.* (2001) found significant associations between maternal blood Pb (mean, 8.9 μg/dL) and childhood blood Pb measures ≤7 years of age with self-report of delinquent behavior in children from the Cincinnati Lead Study evaluated at 15-17 years of age (concurrent mean, 3 μg/dL); however, as in the study by Chen *et al.* (2007), blood Pb levels during early childhood were above 10 μg/dL, so it is not clear that this is strictly associated with blood Pb levels <10 μg/dL. Similar results were reported for umbilical cord blood and concurrent blood Pb and behavior problems in 3-year-olds from the Yugoslavia Prospective Study; however, blood Pb levels (means of 5.5 μg/dL in Pristina and 22 μg/dL in Mitrovica) in many children in this study were also >10 μg/dL (Wasserman *et al.* 1998). Chandramouli *et al.* (2009) found that the odds ratio for antisocial behaviors (OR=2.90 (95% CI: 1.05, 8.03)) in 7- and 8-year-olds (n=582) in the United Kingdom assessed by the Anti-social Behavior Interview was significantly elevated with blood Pb >10 μg/dL at 30 months of age. Two studies have also reported an association with prenatal or childhood blood Pb levels and antisocial behavior problems as young adults[5]. Higher rates for total criminal arrests in 19- to 24-year-olds for a 5 μg/dL increase in blood Pb were significantly associated with higher maternal blood Pb during the first or early second trimester of pregnancy (mean, 8.3 μg/dL; relative risk (RR)=1.4 (95% CI: 1.07, 1.85)) as well as blood Pb at 6 years of age (mean, 8.3 μg/dL; RR=1.27 (95% CI: 1.03, 1.57)) in a study of 250 young adults from the Cincinnati Lead Study (Wright *et al.* 2008). Hornung *et al.* (2009) reported that blood Pb at 6 years of age was more strongly related to adult criminal arrests (age was not reported) than was blood Pb at 2 years of age in a similar analysis reported for the combined cohort from the Cincinnati and Rochester lead studies, with a peak blood Pb mean of 13.6 μg/dL and concurrent blood Pb at 6 years of age of 6 μg/dL; there was a strong correlation between the ratio of blood Pb at 6 years to blood Pb 2 years and criminal arrests (β=1.21; $p<0.001$).

Several studies support an association between higher bone Pb, tooth dentin Pb, or hair Pb and antisocial problem behaviors but did not provide blood Pb measurements for comparison. Bone Pb levels were associated with antisocial behavior, including delinquency and aggression, in a study of 212 boys tested by the CBCL at 7 and 11 years of age (Needleman *et al.* 1996). Needleman *et al.* (2002) found that tibia bone Pb in 12- to 18-year-olds was associated with an increased odds ratio for delinquent behavior that resulted in a court appearance (OR=3.7 (95% CI: 1.3, 10.5)) in a case-control study of 194 delinquent and 146 nondelinquent youths from the same high schools in Pennsylvania. Fergusson *et al.* (2008) reported a significant association between tooth dentin Pb of shed primary teeth (6-8 years of age) and officially reported crime at 21 years of age in a study of 1,265 young adults from the Christchurch Health and Development Study cohort.

[5] Note: children are defined as <18 years of age in this document; therefore, 19- to 24-year-olds in the following studies are considered adults.

A meta-analysis of 19 studies of Pb exposure and conduct problems in children 3-18 years of age reported a significant overall correlation (r=0.19; p<0.001) or medium effect size across the 19 studies evaluated, consisting of 8,561 total children (Marcus *et al.* 2010). Although conduct problems were more common in boys than in girls, the percentage of boys in the study did not appear to attenuate the relationship with Pb, nor did adjustment for other confounders such as age, socioeconomic status, parental IQ, or home environment. Marcus *et al.* (2010) note that effects were similar whether Pb exposure was measured by blood Pb, by bone Pb measured in tooth dentin, or by bone Pb measured with K-x-ray analysis; however, a larger effect of hair Pb was found in three studies from the same laboratory (Marlowe and Errera 1982, Marlowe *et al.* 1985, Marlowe and Bliss 1993) that used hair as the measure of exposure. Marcus *et al.* (2010) cannot explain why the hair Pb data displayed a stronger relationship and noted that multiple studies have determined that hair Pb is less accurate than blood Pb measurements for determining Pb exposure (e.g., ATSDR 2001)(see discussion in **Section 3.2 Biomarkers of Pb Exposure**). This suggests that the Marlowe *et al.* studies contain a bias or other population factor that may explain the stronger relationship in these studies.

Summary of support for conclusions

Animal data support a Pb-associated neurobehavioral deficits (including reduced ability to inhibit inappropriate responding) at blood Pb levels close to levels reported in human studies (i.e., approximately 10 µg/dL) (see U.S. EPA 2006 for recent reviews of the animal data, ATSDR 2007). The human data from multiple prospective and cross-sectional studies support a Pb-associated increase in antisocial problem behaviors or criminal behavior in children from 6 to 15 years of age with concurrent blood Pb levels of <1-15 µg/dL. The conclusion of *sufficient* evidence for a positive association with behavior problems in children at blood Pb levels <5 µg/dL is based on the consistency of effects in these studies and the Braun *et al.* (2008) study from the NHANES 2001-2004 data set that reported conduct disorder at blood Pb levels ≥0.8 µg/dL blood Pb. Most of the recent studies in the database include a large number of confounders, such as socioeconomic variables, sex, race/ethnicity, age of blood Pb measurement, parental education, and tobacco exposure. Several studies reported that blood Pb levels were significantly associated with antisocial behavioral problems even after controlling for child IQ through model adjustments or by path analysis (Silva *et al.* 1988, Burns *et al.* 1999, Chen *et al.* 2007). Although the Wright *et al.* (2008) study reported that criminal arrests in young adults 19-24 years of age were associated with prenatal and childhood blood Pb levels, and the Fergusson *et al.* (2008) study found an association between reported crimes in 21-year-olds and dentin Pb of shed primary teeth at 6-8 years of age, no studies of antisocial behavioral problems were located that used Pb levels in adults. There is *inadequate* evidence to evaluate the potential association between blood Pb <10 µg/dL and effects on behavior problems in adults. The conclusion of *limited* evidence for a positive association between prenatal Pb exposure to blood Pb <10 µg/dL and behavioral problems in children is based on the mixed results of studies with prenatal exposure data. The NTP's conclusion of *sufficient* evidence that antisocial behavior problems in children are associated with Pb levels <5 µg/dL is stronger than the discussion of Pb effects on mood and behavior that may extend into increased risk for delinquent behavior described in the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's 2007 Toxicological Profile for Lead (ATSDR 2007); however, given the growth

of the database in recent years, the EPA's 2012 draft (U.S. EPA 2012) currently supports an association with problem behavior.

Psychiatric Outcomes, Including Anxiety and Depression

There is *inadequate* evidence to evaluate the potential association between blood Pb <10 μg/dL and anxiety- or depression-related psychiatric outcomes in children (see Appendix A: Neurological Effects for full list of studies). There is *limited* evidence that blood Pb <10 μg/dL in adults is associated with psychiatric symptoms that include anxiety and depression. Although relatively few studies of children or adults with low blood Pb levels address these mood symptoms, the available data in adults are from several large cross-sectional studies (n=526-1,987) and support an effect of concurrent blood Pb or bone Pb on these psychiatric outcomes.

Most studies of behavior in children that have identified an association with Pb exposure have been in the area of attention-related behaviors or conduct problems. However, several studies have reported an association between blood Pb at and above 10 μg/dL and anxiety- or depression-related behaviors in children. Wasserman *et al.* (1998) reported that umbilical cord blood Pb (means of 5.5 μg/dL in Pristina and 22 μg/dL in Mitrovica) and concurrent blood Pb levels in 3-year-olds were associated with somatic problems, anxious-depressed and withdrawn behavior by the CBCL in a study of 293 children from the Yugoslavia Prospective Study; however, many children in this study had blood Pb levels above 10 μg/dL, so these effects may not be associated with blood Pb levels <10 μg/dL. Roy *et al.* (2009) reported increased anxiety and social problems evaluated by teachers using the Conners' Teacher Rating Scales in 3- to 7-year-old children in India with concurrent mean blood Pb of 11 μg/dL. In a small study of 42 children 3-5 years of age with a mean blood Pb of 2 μg/dL, higher blood Pb was associated with lower teacher ratings of social confidence and sociability in girls but not to measures of anxiety or aggression in boys or girls (Hubbs-Tait *et al.* 2007).

Several studies have demonstrated an association between concurrent blood Pb <10 μg/dL in adults and psychiatric symptoms, including anxiety, depression, and panic disorder. However, these studies do not include cohorts in which it has been demonstrated that blood Pb levels were consistently below 10 μg/dL from birth to behavioral assessment. Bouchard *et al.* (2009) reported a significant increase in the odds ratio for diagnoses of major depression disorder (OR=2.32 (95% CI: 1.13, 4.75)) and panic disorder (OR=4.94 (95% CI: 1.32, 18.48)) at concurrent blood Pb levels of ≥2.11 μg/dL in a study of 1987 adults 20-39 years of age from the NHANES 1999-2004 data set; a diagnosis of generalized anxiety disorder was not associated with blood Pb levels in this study. In a study of 526 men in the Normative Aging Study (mean age, 67 years), blood Pb (mean, 6.3 μg/dL), tibia Pb (mean, 22 μg/g), and patella Pb (mean, 32 μg/g) were significantly associated with combined measure of mood, including elevated anxiety, depression, and phobic anxiety (Rhodes *et al.* 2003). In a further study of 744 men from the Normative Aging Study, an interquartile increase in tibia bone Pb (14 μg/g) or patella Pb (20 μg/g) was associated with increased risk of psychiatric symptoms of somatization and increased global severity index (Rajan *et al.* 2007).

Summary of support for conclusions

There are some examples of Pb-associated increases in depression-related outcomes in rats and mice at blood Pb levels down to 17 μg/dL (e.g., Dyatlov and Lawrence 2002, U.S. EPA 2006 for recent reviews of the animal data, see ATSDR 2007). The data set of human studies to evaluate the association with psychiatric outcomes is relatively small both for children and for adults; however, several studies in adults support an effect of concurrent blood Pb <10 μg/dL or bone Pb. The conclusion of *inadequate* evidence to evaluate the potential association between blood Pb <10 μg/dL in children and anxiety- or depression-related psychiatric outcomes is based on the lack of studies at blood Pb levels <10 μg/dL. The conclusion of *limited* evidence that concurrent adult blood Pb levels <10 μg/dL are associated with psychiatric symptoms including anxiety and depression is based on the small number of studies supporting an effect (one at blood Pb <10 μg/dL and one at blood Pb <5 μg/dL) and because two of the three studies are from a single cohort, the Normative Aging Study. As with other studies of health effects of Pb in adults, prospective studies in a group for which blood Pb levels remained consistently below 10 μg/dL from birth until assessment of anxiety, depression, or other psychiatric outcomes would eliminate the potential role of early-life blood Pb levels above 10 μg/dL on health effects observed in adults with concurrent blood Pb levels <10 μg/dL. The 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) and 2006 EPA AQCD for Lead (U.S. EPA 2006) discuss evidence that effects of Pb may extend into increased risk for antisocial and delinquent behavior. The 2006 EPA AQCD for Lead (U.S. EPA 2006) does not have specific conclusions on the potential association between Pb exposure and anxiety. The 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) highlights the evidence for neurobehavioral effects in older adults at blood Pb levels >4 μg/dL.

4.3.3 Neurodegeneration

Amyotrophic Lateral Sclerosis (ALS)

There is *limited* evidence that blood Pb levels <10 μg/dL are associated with increased risk for ALS. A number of case-control studies have reported a significant association between blood Pb and ALS diagnosis, with most of the studies coming from two patient groups (see Appendix A: Neurological Effects for full list of studies). The data present limited evidence because of issues with potential reverse causality, because an association between ALS and bone turnover might lead to higher Pb levels among ALS patients, and issues with bias because a reported increased survival time in ALS patients relative to control patients might also lead to higher Pb levels.

In a small case-control study of 19 ALS patients and 39 controls (mean age, 64-66 years), mean blood Pb was not significantly different (p=0.38) between ALS patients (12.7 μg/dL) and controls (10.8 μg/dL) (Vinceti *et al.* 1997). The relative risk of ALS was significantly associated with blood Pb (continuous measure: OR=1.9 (95% CI: 1.4, 2.6); categorical variable, 3-4 μg/dL: OR=14.3 (95% CI: 3, 69)), but the association with bone Pb was not significant, in a case-control study of 109 ALS patients and 256 matched controls in New England (Kamel *et al.* 2002). The study reported that cases had greater odds of having a job with Pb exposure (OR=1.9 (95% CI: 1.1, 3.3)) compared to controls, and odds ratio for having ALS was significantly

associated with lifetime days of Pb exposure greater than 2,000 hours (OR=2.3 (95% CI: 1.1, 4.9)). The significant association with lifetime exposure is interesting because bone Pb was not significantly associated with ALS in the study, and bone Pb is considered a better measure of cumulative exposure. The issue of reverse causality (the possibility that increased blood Pb is related to greater bone Pb mobilization from the reduced physical activity in ALS patients) was examined in a separate case-control study of 184 ALS cases and 194 controls among U.S. veterans (Fang *et al.* 2010). Fang *et al.* (2010) reported that blood Pb was significantly higher among the ALS patients (mean, 2.4 µg/dL) than among controls (1.8 µg/dL). The odds ratio for having ALS was significantly associated with blood Pb (OR=2.6 (95% CI: 1.9, 3.7) for a doubling of blood Pb). The study examined the potential influence of measures of bone turnover and genetic factors and reported a significant interaction between blood Pb – ALS and plasma biomarkers for bone turnover (procollagen type-1 amino-terminal peptide (PINP)) and resorption (C-terminal telopeptides of type 1 collagen (CTX)), but not the K59N polymorphism in the *ALAD* gene (Fang *et al.* 2010). However, adjusting the model to account for differences in biomarkers of bone turnover did alter the association between blood Pb and ALS to a large degree. The authors state that reverse causality is unlikely because the Pb-ALS association persisted after adjusting for biomarkers of Pb mobilization from bone, but that reverse causality cannot be entirely ruled out because of the cross-sectional nature of the data.

In further study of the population in New England, Kamel *et al.* (2003, 2005) reported that the *ALAD* gene was associated with altered bone Pb levels, but not with blood Pb, and the effect on ALS was not significant. In a second, follow-up study, Kamel *et al.* (2008) demonstrated that tibia bone Pb was significantly associated with greater survival time between diagnosis and death (HR=0.3 (95% CI: 0.1, 0.7)), while the association between greater survival time and patella Pb (HR=0.5 (95% CI: 0.2, 1.0)) or blood Pb (HR=0.9 (95% CI: 0.8, 1.0)) had only borderline significance. The association of bone and blood Pb with greater survival time in ALS patients relative to control patients introduces the possibility of bias, because the case groups in these case-controls studies may be individuals with longer survival time, and higher Pb levels may be related to a longer period of exposure or more time for Pb to be released from bone stores.

Summary of support for conclusions

Several recent animal studies have demonstrated findings similar to the human data supporting a relationship between Pb and ALS. For example, Barbeito *et al.* (2010) reported that blood Pb levels of 27 µg/dL were associated with increased survival time in a mouse model of severe ALS, analogous to the longer survival time in ALS patients with higher blood Pb levels observed by Kamel *et al.* (2008). The NTP concluded that there is *limited* evidence that blood Pb levels <10 µg/dL are associated with diagnosis of ALS because the case-control studies that reported an association with blood Pb have potential issues with reverse causality and bias due to a reported increased survival time in ALS patients relative to control patients, both of which might also lead to higher Pb levels in ALS patients. The data from Fang *et al.* (2010) addressed some of the reverse causality issues by controlling for factors associated with bone turnover. As with other studies of health effects of Pb in adults, studies that demonstrate an association between ALS and blood Pb in a group for which blood Pb levels remained consistently below 10 µg/dL from birth until diagnosis of ALS would eliminate the potential role of early-life blood

Pb levels above 10 µg/dL on health effects observed in adults with concurrent blood Pb levels <10 µg/dL. The NTP's conclusions for *limited* evidence that blood Pb levels <10 µg/dL are associated with diagnosis of ALS is consistent with the four studies highlighted in EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's 2007 Toxicological Profile for Lead (ATSDR 2007).

Alzheimer's disease

There is *inadequate* evidence to evaluate the potential association between blood Pb <10 µg/dL and Alzheimer's disease because no studies examining Alzheimer's disease in groups with blood Pb levels <10 µg/dL were located. Although the ATSDR's 2007 Toxicological Profile for Lead (ATSDR 2007) and EPA's 2006 AQCD for Lead (U.S. EPA 2006) have similar conclusions on the lack of epidemiological evidence for an association between Pb and Alzheimer's disease, the EPA's 2006 AQCD for Lead (U.S. EPA 2006) highlights the evidence from studies of laboratory animals that early-life exposure to Pb is associated with Alzheimer-like pathologies at later ages. In particular, exposure of rats (Basha *et al.* 2005) and monkeys (Zawia and Basha 2005) to Pb during development was associated with overexpression of the amyloid precursor protein associated with Alzheimer's disease; however, Pb exposure in adult animals did not result in increased amyloid deposits in the brain. It should also be noted that laboratory animal data have not demonstrated dementia in existing models.

Essential tremor

There is *sufficient* evidence that blood Pb levels <10 µg/dL and *limited* evidence that blood Pb levels <5 µg/dL in adults are associated with increased risk for diagnosis of essential tremor, a type of involuntary tremor. A number of case-control studies have reported a significant association between blood Pb and essential tremor diagnosis, with most of the studies coming from New York and similar results reported for a population in Turkey (see Appendix A: Neurological Effects for full list of studies). The data present consistent evidence for an association between blood Pb and essential tremor from two distinct groups in different countries, but reflect a total sample size of only approximately 300 cases of essential tremor.

Louis *et al.* (2003) reported that blood Pb was significantly higher in essential tremor patients (3.3 µg/dL) than in controls (2.7 µg/dL) in a case-control study of 100 essential tremor cases (mean age, 66 years) and 143 matched controls (mean age, 71 years) from New York. Blood Pb was significantly associated with an increased odds ratio for essential tremor diagnosis OR=1.21 (95% CI: 1, 1.39). Louis *et al.* (2005, 2011) also examined the interaction between blood Pb and other modifying factors such as *ALAD* genetic polymorphisms and exposure to harmane (a β-carboline alkaloid compound associated with essential tremor) in this New York cohort. The odds of essential tremor were significantly, and to a large degree, elevated in individuals with the ALAD-2 allele and higher blood Pb (OR=80 (95% CI: 3, 2096)) in a case-control study of 63 essential tremor cases and 101 matched controls (Louis *et al.* 2005). In a further case-control study of 106 essential tremor cases and 151 controls, Louis *et al.* (2011) found that essential tremor score was highest in individuals with higher concentrations of both Pb and harmane; they concluded that there was an additive effect of Pb and harmane on essential tremor severity. Louis collaborated with researchers in Turkey to examine essential tremor and Pb in Turkey, a population distinct from the New York-based group in earlier

publications. In a case-control study in 105 essential tremor patients and 105 controls from Turkey, Dogu *et al.* (2007) reported that blood Pb (mean, 3.2 µg/dL in cases and 1.6 µg/dL in controls) was associated with a significantly greater odds ratio for essential tremor diagnosis (OR=4.19 (2.59,6.78)).

Summary of support for conclusions

Animal data support Pb-associated neurological effects, including tremor, at higher Pb exposure levels (e.g., Booze *et al.* 1983, see U.S. EPA 2006, ATSDR 2007 for recent reviews of the animal data). The NTP concluded that there is *sufficient* evidence that blood Pb levels <10 µg/dL and *limited* evidence that blood Pb levels <5 µg/dL are associated with diagnosis of essential tremor. The data are considered to provide *sufficient* evidence because the case-control studies that reported an association with blood Pb are from two distinct, widely separated groups which report the same pattern of effects. Given that the two studies represent a small total number of essential tremor patients (sample size of about 300), a conclusion of *limited* evidence at the lower blood Pb level <5 µg/dL is supported. As with other studies of health effects of Pb in adults, prospective studies in a group for which blood Pb levels remained consistently below 10 µg/dL from birth until diagnosis of essential tremor would eliminate the potential role of early-life blood Pb levels >10 µg/dL on health effects observed in adults with concurrent blood Pb levels <10 µg/dL. The NTP's conclusions for *sufficient* evidence that blood Pb levels <10 µg/dL and *limited* evidence that blood Pb levels <5 µg/dL are associated with diagnosis of essential tremor is consistent with the blood Pb level of 3 µg/dL highlighted in the two studies listed in EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's 2007 Toxicological Profile for Lead (ATSDR 2007).

Parkinson's disease

There is *inadequate* evidence to evaluate the potential association between blood Pb <10 µg/dL and Parkinson's disease, because few studies examining this association were located, and no studies were identified in groups with blood Pb levels <10 µg/dL. Two studies were found that reported a significant association between lifetime Pb or bone Pb and incidence of Parkinson's disease. In a case-control study of 121 Parkinson's disease patients and 414 controls in Michigan, Coon *et al.* (2006) reported that the odds ratio of a diagnosis of Parkinson's disease was increased for whole-body lifetime Pb exposure (blood and bone combined, by a physiologically based pharmacokinetic model) and was significantly elevated in the top fourth (highest quartile) of Pb measurements (OR=2.27 (95% CI: 1.13, 4.55)), but there was no association with bone Pb alone. Weisskopf *et al.* (2010) reported that the odds ratio for Parkinson's disease was increased (OR=3.21 (95% CI: 1.17, 8.83)) for highest quartile of tibia Pb compared to the lowest quartile in a case-control study of 330 Parkinson's disease patients and 308 controls in Boston.

4.3.4 Sensory organs

Auditory

There is *sufficient* evidence that blood Pb levels <10 µg/dL in children are associated with decreased hearing (decreased auditory acuity). Cross-sectional studies reported a Pb-related

increase in hearing thresholds and an increase in latency of BAEPs in children and young adults[6] 4-19 years of age, and altered BAEPs in newborns (see Appendix A: Neurological Effects for full list of studies). Hearing loss indicated by higher hearing thresholds have been demonstrated at blood Pb levels <10 μg/dL, and increased BAEP latency has been reported at slightly higher blood Pb levels (<10 μg/dL). There is *limited* evidence that prenatal blood Pb levels <10 μg/dL are associated with auditory effects because few studies have addressed low-level Pb exposure during this developmental period. There is also *limited* evidence that blood Pb levels <10 μg/dL in adults are associated with decreased auditory acuity due to the limited number of studies with blood Pb data <10 μg/dL and auditory effects in adults.

In a cross-sectional study of 4,519 children and young adults 4-19 years of age from the NHANES II data set, Schwartz and Otto (1987) reported a significant association between blood Pb level and hearing loss as determined by an increase in hearing thresholds for pure-tone frequencies from 500 to 4,000 Hz. In a second study using the same measures of hearing, Schwartz and Otto (1991) found that mean hearing thresholds were significantly increased in association with blood Pb levels in a large cross-sectional study of 3,545 children and young adults 6-19 years of age from the Hispanic Health and Nutrition Examination Survey. Hearing loss was observed at blood Pb levels ≥8 μg/dL, and a 2-decibel hearing loss at all frequencies was reported with an increase in blood Pb from 6 to 18 μg/dL (Schwartz and Otto 1991). Similar results were observed in a study of 155 Polish children 4-14 years of age, with hearing loss demonstrated by increased hearing thresholds at blood Pb levels <10 μg/dL (median blood Pb, 7.2 μg/dL); there were also increased latencies of peak I BAEPs that were significant for blood Pb levels >10 μg/dL compared to children with blood Pb <4.6 μg/dL (Osman *et al.* 1999). Increased latencies of BAEP waves I-IV have also been reported at higher blood Pb levels (10 μg/dL) in adults and children (reviewed in Otto and Fox 1993), and in two studies of children with higher mean blood Pb that included some subjects with blood Pb levels <10 μg/dL (Otto *et al.* 1985, Robinson *et al.* 1985). An effect of prenatal exposure is supported by data from the Rothenberg *et al.* (1994, 2000) studies demonstrating that the latency and interpeak interval of BAEPs were significantly altered in infants (n=30 born to mothers with pregnancy Pb levels of 2.5-35 μg/dL) and 5- and 6-year-olds (n=100) born to mothers with mean blood Pb levels of 8 μg/dL. Dietrich *et al.* (1992) reported that performance on the screening test for auditory processing disorders at 5 years of age was inversely affected in a study of 259 children from the Cincinnati Lead Study with mean prenatal blood Pb 8 μg/dL, and infant blood Pb of 5 μg/dL; however, mean blood Pb levels from 1 to 5 years of age ranged from 10 to 17 μg/dL, so effects may not be associated with blood Pb levels <10 μg/dL.

Four studies in adults addressed individuals with lower blood Pb levels. Forst *et al.* (1997) reported that blood Pb level (mean, 5 μg/dL; range, 1-18 μg/dL) was associated with an elevated hearing threshold at 4,000 Hz, but not at other frequencies, examined in a study of 183 workers. Hwang *et al.* (2009) found that hearing thresholds were significantly increased in a study of 259 steel plant workers in Taiwan at blood Pb levels ≥7 μg/dL (mean blood Pb,

[6] Note: children are defined as <18 years of age in this document; therefore, 18- and 19-year-olds in the study are considered adults.

5 µg/dL) for frequencies from 3,000 to 8,000 Hz but not for lower frequencies. In a case-control study of 121 adult cases referred for hearing testing with geometric mean blood Pb 10.7 µg/dL and 173 workers with normal hearing (mean blood Pb, 4 µg/dL), blood Pb was significantly associated with higher hearing thresholds (Chuang *et al.* 2007). In a cross-sectional analysis of 448 men in the Normative Aging Study (mean age, 65 years at time of bone Pb measurement), tibia Pb (mean, 23 µg/g) and patella Pb (mean, 33 µg/g) were significantly associated with hearing loss indicated by higher hearing thresholds at 2,000-8,000 Hz and pure-tone averages (Park *et al.* 2010). Although blood Pb and the potential relationship between blood Pb and hearing were not examined in the Park *et al.* (2010) study, other studies reported mean concurrent blood Pb levels in members of this cohort as <10 µg/dL (Rajan *et al.* 2007). Additional support for effects in adults is provided by the large cross-sectional studies of individuals from 4 to 19 years of age (Schwartz and Otto 1987, 1991) described earlier; these studies included young adults in the age range of the group studied (i.e., individuals 18 and 19 years of age are considered adults).

Summary of support for conclusions

Animal data support a Pb-associated effect on auditory acuity determined by an increase in the latency of BAEPs at blood Pb levels higher than the level observed in human studies (i.e., 33-100 µg/dL) (see U.S. EPA 2006 for recent reviews of the animal data, ATSDR 2007). The human data from several cross-sectional and prospective studies support a Pb-associated decrease in auditory acuity and increase in latency of BAEPs in children with blood Pb levels <10 µg/dL. The conclusion of *sufficient* evidence for decreased auditory acuity at concurrent blood Pb levels <10 µg/dL in children is based on the consistency of effects between hearing loss as determined by increased hearing thresholds and increased latency of BAEPs. In adults, the conclusion of *limited* evidence for similar effects at concurrent blood Pb levels <10 µg/dL is based on the small number of studies supporting an effect (two at blood Pb <10 µg/dL with total n <450), the two Schwartz and Otto (1987, 1991) cross-sectional studies that included adults 18 and 19 years of age in studies primarily focused on children, and supporting evidence from occupational studies at higher blood Pb levels (e.g., Bleecker *et al.* 2003) plus additional supporting evidence of an effect of Pb from bone Pb data in a group of elderly men. As with other studies of health effects of Pb in adults, prospective studies in a group for which blood Pb levels remained consistently below 10 µg/dL from birth until measurement of auditory acuity would eliminate the potential role of early-life blood Pb levels above 10 µg/dL on auditory effects observed in adults with concurrent blood Pb levels <10 µg/dL. The conclusion of *limited* evidence that prenatal exposure to blood Pb <10 µg/dL is associated with auditory effects is based on the two Rothenberg *et al.* (1994, 2000) studies and the Dietrich *et al.* (1992) study that demonstrated an effect of maternal exposure but provided data only on 100 individuals with blood Pb <10 µg/dL that remained <10 µg/dL until auditory function was tested. The NTP's conclusion for *sufficient* evidence that blood Pb levels <10 µg/dL are associated with decreased auditory acuity in children is in line with the supportive evidence of a relationship with auditory processing decrements outlined in EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's 2007 Toxicological Profile for Lead (ATSDR 2007).

Visual

There is *inadequate* evidence to evaluate the potential association between blood Pb <10 µg/dL and effects on vision in children or adults. Multiple studies reported an inverse relationship between blood Pb levels ≤10 µg/dL and visual-motor performance evaluated with tests such as the Beery Developmental Test (e.g., Wasserman *et al.* 2000, Al-Saleh *et al.* 2001, Chiodo *et al.* 2004); however, few epidemiological studies addressed the effects of low-level Pb exposure on visual function. Only three studies were located that examined the impact of blood Pb levels <10 µg/dL and retinal or visual function. Maternal blood Pb at 12 weeks of pregnancy (mean, 8.5 µg/dL) in 45 participants of the Mexico City Prospective Study was associated with altered retinal function in 7- to 10-year-old children, as indicated by changes in electroretinographic (ERG) testing results (Rothenberg *et al.* 2002). In a study of 100-200 children in Artic Quebec at 5 and 11 years of age, Boucher *et al.* (2009) demonstrated that umbilical cord blood Pb (mean, 5 µg/dL) was significantly associated with changes in visual brain signals (event-related potential P3b wave amplitude) at 5 years but not at 11 years of age. Altmann *et al.* (1998) reported that blood Pb levels (mean, 4 µg/dL) in 6-year-old children (n= 384) in Germany were associated with altered visual function as determined by changes in interpeak latency of VEPs. Animal data include evidence for retinal and visual cortical structural and functional abnormalities in rats and nonhuman primates at 11-300 µg/dL (reviewed in Otto and Fox 1993, U.S. EPA 2006, ATSDR 2007, Fox and Boyles 2007). Recent studies in rats support the limited data in humans, and rats exposed to Pb also displayed significant changes in ERG testing results (Fox *et al.* 2008). The NTP concludes that there is *inadequate* evidence to evaluate the potential association between blood Pb <10 µg/dL and effects on vision because of the general lack of human data on retinal or visual function in individuals with blood Pb levels <10 µg/dL. Increased latency in VEPs has been demonstrated in studies of adults with higher blood Pb levels (e.g., 60 down to 17µg/dL in Abbate *et al.* 1995). The report of a potential lower threshold of 14 µg/dL for postural sway in adults with higher occupational Pb exposure (Iwata *et al.* 2005) provides some support for an effect on the auditory and visual systems, because postural sway requires the integration of visual and vestibular input along with peripheral sensory input and motor output. The NTP's conclusions for *inadequate* evidence that blood Pb levels <10 µg/dL are associated with effects on vision in humans are in line with the supportive evidence of a relationship with auditory processing decrements outlined in the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's 2007 Toxicological Profile for Lead (ATSDR 2007) and identification of a potential threshold of 14-20 µg/dL for effects including VEPs in adults.

4.4 Conclusions

The NTP concludes there is *sufficient* evidence that blood Pb levels <5 µg/dL are associated with neurological effects in children and *limited* evidence that blood Pb levels <10 µg/dL are associated with adverse neurological effects in adults (see **Table 4.3: NTP conclusions on neurological effects of low-level Pb** for complete list of conclusions). A major strength of the evidence for effects of low-level Pb on neurological outcomes is in the consistency of results for an adverse effect of blood Pb <10 µg/dL across multiple indices of neurological effects (e.g., cognition, behavior, and sensory function), through multiple groups, with a wide age range from early childhood to older adults, and from studies using substantially different methods

and techniques. In some studies, blood Pb levels of 2 µg/dL are associated with effects in children (e.g., academic achievement in Miranda *et al.* 2007, ADHD in Cho *et al.* 2010). In children, there is *sufficient* evidence that blood Pb levels <5 µg/dL are associated with various indices of reduced cognitive function, increased incidence of attention-related behavior diagnosis, and increased behavioral problems, and there is *sufficient* evidence that blood Pb levels of 10 µg/dL are associated with decreased auditory function. In adults, there is *sufficient* evidence that blood Pb levels <10 µg/dL and *limited* evidence that blood Pb levels <5 µg/dL are associated with essential tremor. There is also *limited* evidence that blood Pb levels <10 µg/dL in adults are associated with psychiatric outcomes, including anxiety and depression, as well as decreases in auditory function, decreases in specific measures of cognitive function in older adults, and the neurodegenerative disease ALS. There are more consistent associations between bone Pb and decreases in cognitive function in older adults than for blood Pb, suggesting a role for cumulative Pb exposure in Pb-related cognitive decline.

Table 4.3: NTP conclusions on neurological effects of low-level Pb				
Health Effect	**Population or Exposure Window**	**NTP Conclusion**	**Blood Pb Evidence**	**Bone Pb Evidence**
Cognitive Function: Academic achievement	Prenatal	*Inadequate*	No studies located	Not studied
	Children	*Sufficient*	Yes, <5 µg/dL	Yes, tooth dentin Pb
Cognitive Function: IQ	Prenatal	*Limited*	Yes, <10 µg/dL	Not studied
	Children	*Sufficient*	Yes, <5 µg/dL	Yes, tibia and tooth dentin Pb
Cognitive Function: Other general and specific measures	Prenatal	*Limited*	Yes, <5 µg/dL	Not studied
	Children	*Sufficient*	Yes, <5 µg/dL	Yes, tibia and tooth dentin Pb
	Older adults	*Limited*	Yes, <10 µg/dL	Yes, tibia and patella Pb
Behavior: Attention-related behaviors	Prenatal	*Limited*	Yes, <10 µg/dL	Not studied
	Children	*Sufficient*	Yes, <5 µg/dL	Yes, tibia and tooth dentin Pb
	Adults	*Inadequate*	No studies located	Not studied
Behavior: Behavioral problems	Prenatal	*Limited*	Yes, <10 µg/dL	Not studied
	Children	*Sufficient*	Yes, <5 µg/dL	Yes, tooth dentin Pb, bone, hair
	Adults	*Inadequate*	No studies located	Not studied
Psychological Effects: Depression, anxiety, other	Prenatal	*Inadequate*	No studies located	Not studied
	Children	*Inadequate*	Unclear, some data >10 µg/dL	Not studied
	Adults	*Limited*	Yes, <10 µg/dL	Tibia and patella Pb
Neurodegeneration: ALS	Adults	*Limited*	Yes, <10 µg/dL	Yes, tibia and patella
Neurodegeneration: Alzheimer's disease	Adults	*Inadequate*	No studies <10 µg/dL located	Not studied
Neurodegeneration: Essential tremor	Adults	*Sufficient*	Yes, <10 µg/dL	Not studied
		Limited	Yes, <5 µg/dL	Not studied
Neurodegeneration: Parkinson's disease	Adults	*Inadequate*	No studies <10 µg/dL located	Yes, tibia and PBPK (cumulative)
Sensory Function: Auditory	Prenatal	*Limited*	Yes, <10 µg/dL	Not studied
	Children	*Sufficient*	Yes, <10 µg/dL	Not studied
	Adults	*Limited*	Yes, <10 µg/dL	Yes, tibia and patella
Sensory Function: Visual	Prenatal	*Inadequate*	Yes, <10 µg/dL	Not studied
	Children	*Inadequate*	Yes, <10 µg/dL	Not studied
	Adults	*Inadequate*	No studies <10 µg/dL located	Not studied

Abbreviation: ALS, amyotrophic lateral sclerosis; PBPK, physiologically based pharmacokinetic.

5.0 IMMUNE EFFECTS

5.1 Conclusions:

The NTP concludes that there is *limited* evidence that blood Pb levels <10 µg/dL are associated with adverse immune effects in children and that there is *inadequate* evidence in adults.

In children, there is *limited* evidence that blood Pb levels <10 µg/dL are associated with increased hypersensitivity responses and allergic sensitization diagnosed by skin prick testing to common allergens. Five studies with mean blood Pb levels of 10 µg/dL and below support the relationship between blood Pb and increased serum IgE (see **Table 5.3**). Two of these studies report an association at blood Pb levels of 10 µg/dL or above, rather than <10 µg/dL (Lutz *et al.* 1999, Sun *et al.* 2003). Only one of the remaining studies, Karmaus *et al.* (2005), considers age as a co-factor in the analyses of IgE (Karmaus *et al.* 2005, Hon *et al.* 2009, Hon *et al.* 2010, Hon 2011). Given these limitations, and particularly the known age-dependent ranges for IgE, these studies provide the basis for the conclusion of *limited* evidence that blood Pb levels <10 µg/dL are associated with elevated serum IgE in children up to 17 years of age. Although increases in serum levels of total IgE do not equate to disease, elevated levels of IgE are the primary mediators of type I hypersensitivity associated with allergic sensitization, and the data demonstrating Pb-related increases in IgE support an association with hypersensitivity. Further support of an association between blood Pb levels <10 µg/dL and hypersensitivity is provided by a prospective study on Pb-related increased allergic sensitization demonstrated by positive response to skin prick testing to common allergens. Together these data support the conclusion of *limited* evidence that blood Pb levels <10 µg/dL are associated with increased hypersensitivity. However, there is *inadequate* evidence of an association between blood Pb and other allergic diseases such as eczema or asthma.

There is *inadequate* evidence in adults to address the potential association between blood Pb <10 µg/dL and IgE, allergy, eczema, or asthma. Few studies have investigated the relationship between immune function and Pb in adults or children, and most studies report general observational markers of immunity rather than function. There is *inadequate* evidence that blood Pb levels <10 µg/dL are associated with observational immune endpoints such as altered lymphocyte counts or serum levels of IgG, IgM, or IgA in the blood of children or adults because of a general lack of studies at the lower dose and inconsistency in available data. There is *inadequate* evidence that blood Pb levels <10 µg/dL are associated with changes in immune function other than hypersensitivity, because there are few studies of Pb and immune function in humans, particularly at lower blood Pb levels. Very few studies examine markers of exposure other than blood Pb levels, and therefore it is unknown if blood or bone Pb levels would be more consistently associated with immune effects.

5.2 How Conclusions Were Reached

Conclusions in the NTP's evaluation of Pb-related immunological effects in humans associated with low-level Pb are derived by evaluating the data from epidemiological studies with a focus on blood Pb levels <10 µg/dL. Although there is a large database of immune studies of Pb in

laboratory animals, the database of human studies is somewhat limited, particularly at blood Pb levels <10 µg/dL. The NTP's conclusions are based on the evidence from human studies with blood Pb levels of <10 µg/dL, with data reflecting exposure levels up to 15 µg/dL also considered so that effects at and around 10 µg/dL were not excluded from the evaluation. Given the limited database of human studies available to evaluate immune effects associated with blood Pb levels <10 µg/dL, a discussion of immune effects associated with higher blood Pb levels is also included in the evaluation. The discussion below also assesses the biological plausibility and support for Pb-associated immune effects provided by the database of studies in laboratory animals. Major endpoints considered as potential indicators of effects of Pb on the immune system are listed and briefly described in **Section 5.2.1**. This document is not a review of the immune system or immunotoxicity, and the reader is directed to published reviews for additional background. Key data and principal studies considered in developing the NTP's conclusions are discussed in detail in **Section 5.3 Evidence for Pb-related Immune Effects.** The discussion of each immune effect begins with a statement of the NTP's conclusion that the specific effect is associated with a blood Pb level <10 µg/dL or <5 µg/dL and the age group in which it is identified (childhood or adulthood), as well as the timing of exposure associated with the effect (prenatal, childhood, concurrent) when available. Although the information necessary to support the NTP's conclusions is presented in **Section 5.3**, the complete data set of human studies considered for evaluation of immune effects with low-level Pb is included in Appendix B: Immune Effects, and individual studies are abstracted for further reference. The NTP made extensive use of recent government evaluations of health effects of Pb in the current assessment, and the relevant conclusions of the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) are briefly described in **Section 5.2.2**.

5.2.1 Principal Measures of Immune Effects

Table 5.1 lists a number of key immune endpoints potentially evaluated in epidemiological studies. A distinction is made between observational markers, which generally have less predictive value for immunotoxicity, and markers of immune function, which are considered better indicators of potential adverse immune effects. Functional assays can be performed in humans, including specific antibody response to

Table 5.1: Major immune effects considered

Effect	Description
Observational:	
Immunoglobulin (Ig) or antibodies	Serum IgE, IgM, IgG; IgA, and IgD (A and D not routinely measured)
Immunophenotyping	White blood cell differential (T-cells, B-cells, NK-cells, monocytes/macrophages, etc.)
Functional:	
Antibody response	Production of Ig to challenge: **Hypersensitivity** evaluated with antigen-specific IgE and skin prick test (SPT) **Suppression** commonly evaluated by specific IgM or IgG after T-cell antigen challenge
Delayed-type hypersensitivity (DTH) response	Type 1 helper T cells and macrophage-dependent DTH response to antigen challenge
Neutrophils	Peripheral blood mononuclear leukocyte phagocytosis, respiratory burst, migration
Monocyte/macrophages	Phagocytosis, respiratory burst (monocytes circulate and mature into tissue macrophages)
Allergy, asthma, eczema, etc.	Clinical manifestation of hypersensitivity

vaccination, delayed-type hypersensitivity (DTH) response, phagocytic activity of neutrophils and macrophages, and oxidative burst of neutrophils and macrophages (Tryphonas 2001). However, human epidemiological data are much more likely to be restricted to observational data, such as circulating immunoglobulin levels, lymphocyte counts, and cytokine levels. The data available to evaluate each of the major effects are discussed in separate subheadings under **Section 5.3** below.

5.2.2 Principal Conclusions from the 2006 EPA and 2007 ATSDR Pb Documents

The EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) both list a number of immune parameters (see **Table 5.2** for principal conclusions and original documents for complete conclusions) that have been reported as altered in populations exposed to Pb, including increased serum IgE levels, altered T-cell and B-cell numbers, changes in macrophage and neutrophil activation, suppressed neutrophil chemotaxis, and phagocytosis. The 2006 EPA AQCD for Lead (U.S. EPA 2006) states that studies have consistently found consistent evidence of increased serum IgE levels in children at blood Pb <10 µg/dL but that results from studies in adults are mixed. The 2006 EPA AQCD for Lead (U.S. EPA 2006) also states that the principal functional immune changes associated with Pb exposure are (1) increases in type 2 helper T-cell (Th-2)- associated production of IgE; (2) suppressed type 1 helper T-cell (Th-1) responses (i.e., DTH); (3) shifting the balance of Th-1/Th-2 cytokines toward a Th-2 response; and (4) stimulating macrophages into a hyper-inflammatory state. The EPA notes that functional changes had not been rigorously evaluated in human studies at the time of the 2006 AQCD for Lead (U.S. EPA 2006), and that the available epidemiological studies rely primarily on observational data detailing circulating immunoglobulin levels and lymphocyte counts. The EPA is in the process of revising the AQCD, and the conclusions of the external draft (U.S. EPA 2012) are largely in line with the 2006 AQCD for Lead.

Table 5.2: Main conclusions for immunological effects in the 2006 EPA AQCD for Lead and the 2007 ATSDR Toxicological Profile for Lead

"…effects include changes in serum immunoglobulin levels; perturbation of peripheral lymphocyte phenotype profiles, including decreases in peripheral blood T-cell abundance and changes in T-cell to B-cell abundance ratios; suppression of lymphocyte activation; and suppression of neutrophil chemotaxis and phagocytosis. Studies of biomarkers of humoral immunity in children have consistently found significant associations between increasing blood Pb concentrations and serum IgE levels at blood Pb levels <10 µg/dL." (U.S. EPA 2006, pg 6-272)
"Altered immune parameters have been described in lead workers with PbB [blood Pb level] in the range of 30-70 µg/dL. Reported effects included changes in some T-cell subpopulations, response to T-cell mitogens, and reduced chemotaxis of polymorphonuclear leukocytes. Several studies of children reported significant associations between PbB and increases in serum IgE levels…" (ATSDR 2007, pg 22)

The NTP considered the conclusions and data summaries from the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007). In general, the NTP concurred with the conclusions and agreed that the data support them. Differences from the ATSDR and EPA documents are identified for specific endpoints in the document.

5.3 Evidence for Pb-related Immune Effects

5.3.1 Increased Serum IgE and Allergic Sensitization

There is *limited* evidence that blood Pb levels <10 µg/dL are associated with increased serum IgE in children up to 17 years of age (**Table 5.3** and Appendix B: Immune Effects). Elevated serum IgE was reported with Pb exposure in five cross-sectional studies involving children from multiple groups in North America, Europe, and China. An association between increased blood Pb and increased IgE has been observed in children at mean blood Pb values from 1.9 to 14 µg/dL and subjects ranging from 1 month to 17 years of age. Three of the five available studies (Karmaus *et al.* 2005, Hon *et al.* 2009, Hon *et al.* 2010, Hon 2011) report an association between blood Pb levels <10 µg/dL and increased serum IgE, but only one of the studies (Karmaus *et al.* 2005) reports an association with blood Pb levels <10 µg/dL in an analyses that adjusted for age, an important confounder when considering IgE levels, particularly in children. The database for potential association between blood Pb and IgE in children is restricted to cross-sectional studies and therefore the data only inform the association between current blood Pb and current IgE. The influence of prenatal Pb exposure on IgE was the subject of a single study (Annesi-Maesano *et al.* 2003) that demonstrated an association between increased IgE and increased infant hair Pb levels but provided equivocal data on the association between maternal blood Pb and infant IgE at birth. Although increases in serum levels of total IgE do not equate to disease, elevated IgE is the primary mediator of type I hypersensitivity associated with allergic sensitization and allergic diseases such as asthma (Beeh *et al.* 2000, Ahmad Al Obaidi *et al.* 2008). The data supporting Pb-related increases in IgE, together with a prospective study on allergic sensitization (diagnosed by skin prick testing), provide *limited* evidence that blood Pb <10 µg/dL is associated with increased hypersensitivity responses in children. However, there is *inadequate* evidence of an association between blood Pb and eczema or asthma in children because the results of the few available studies are generally negative for asthma and because the eczema data come from a pair of studies of a single group of children from a dermatology clinic. There are fewer studies of the association between low level Pb (blood <10 µg/dL) and hypersensitivity in adults than for children, and available data that address IgE and related endpoints are mixed. There is *inadequate* evidence in adults to address the potential association between blood Pb <10 µg/dL and IgE, allergy, eczema, or asthma.

Increased serum IgE was reported in five cross-sectional studies of children from 1 month to 17 years of age and mean blood Pb from 1.9 to 14 µg/dL (Lutz *et al.* 1999, Sun *et al.* 2003, Karmaus *et al.* 2005, Hon *et al.* 2009, Hon *et al.* 2010, Hon 2011). Higher serum IgE correlated (r=0.22; p=0.0004) with higher blood Pb levels in a study of 279 children in Missouri 9 months to 6 years of age (Lutz *et al.* 1999). Lutz *et al.* (1999) stated that 64% of the children in their study had blood Pb <10 µg/dL; however, the authors do not report mean blood Pb levels and therefore the data may reflect an effect of blood Pb levels above 10 µg/dL. Serum IgE was also correlated with blood Pb levels (r=0.48; p=0.002) in children with blood Pb levels ≥10 µg/dL in a subsample of 72 children with a mean blood Pb level of 14 µg/dL from a larger study of 217 children 3-6 years of age with overall mean blood Pb of 9.5 µg/dL in China (Sun *et al.* 2003). Although the Lutz *et al.* (1999) and Sun *et al.* (2003) studies report an effect of blood Pb at levels near

Table 5.3: Studies of serum IgE, sensitization, and eczema with low-level Pb used to develop conclusions for children				
Relevance to Conclusions	**Study Description**	**Study Design**	**Key Immunological Findings**	**Reference**
Increased IgE				
Effect	374 newborns in Paris Study A: 1985 Study B: 1991-1992	Cross-sectional	**Increased serum IgE** in umbilical cord blood was associated with infant hair levels of Pb. IgE was also associated with maternal blood Pb in study B (Pb=6 µg/dL) not in study A (Pb=13 µg/dL) or with infant blood in either study.	Annesi-Maesano (2003)
Effect	331 children 7-10 years old in Germany	Cross-sectional	**Increased serum IgE** was associated with current blood Pb (mean Pb, 2.7 µg/dL).	Karmaus (2005)
Equivocal	318 children 0.5-7 years old in Egypt	Cross-sectional	**Serum IgE** differed by blood Pb (mean Pb, 9.2 µg/dL); however, authors report that IgE is not correlated with blood Pb.	Hegazy (2011)
Effect	279 children 0.75-6 years old in Missouri	Cross-sectional	**Increased serum IgE** was associated with current blood Pb (mean not reported, 64% had blood Pb <10 µg/dL).	Lutz (1999)
Effect	72 children aged 3-6 in China of 217 in study	Cross-sectional	**Increased serum IgE** was correlated with blood Pb in children with Pb ≥10 µg/dL. Increased serum IgE in girls with blood Pb≥10µg/dL relative to Pb <10 µg/dL; not boys.	Sun (2003)
Hong Kong Eczema patents		Cross-sectional	**Increased serum IgE** was positively correlated with blood Pb (mean Pb, 1.9 µg/dL) in children with eczema (age > 1 month; mean age, 10 years).	Hon (2010, 2011); may overlap with Hon (2009)
Effect	110 children age ≤17			
Effect	58 children age 10	Cross-sectional	**Increased serum IgE** was positively correlated with blood Pb (mean Pb, 1.9 µg/dL) in children with eczema (age > 1 month; authors state average age of 10 years).	Hon (2009) *Also for eczema*
Effect	2,470 children aged 5-14 in Germany	Ecological	**Odds ratios for increase in specific IgE** to common allergens were elevated in children from a polluted area in Germany that had higher Pb dustfall; no blood Pb data.	Heinrich (1999) *Also for sens. & eczema*
Evidence of enhanced sensitization based on skin prick test (SPT)				
Effect	224 children 5 years old in Poland	Prospective	Frequency of atopy (positive SPT) associated with cord Pb (mean, 1.2 µg/dL) and maternal blood Pb (mean, 1.6 µg/dL); not current Pb. Risk ratio for SPT/atopy related to cord Pb; authors state that prenatal Pb may enhance sensitization to aeroallergens.	Jedrychowski (2011)
Effect	2,470 children aged 5-14 in Germany	Ecological	Odds ratio for sensitization (positive SPT) or allergy (doctor diagnosis) was elevated in children from a polluted area in Germany that had higher Pb dustfall; no blood Pb data.	Heinrich (1999) *Also for IgE and eczema.*
Eczema and atopic dermatitis				
No effect	1,768 children born in Boston 1979-1981	Retrospective	Relative risk of eczema in childhood (age not reported) did not differ for children with umbilical cord blood Pb >10 µg/dL compared to rest of the population.	Rabinowitz (1990)
Hong Kong Eczema patents		Cross-sectional	Atopic dermatitis severity, eczema severity score, and children's dermatology life quality index were positively correlated with blood Pb (mean, 1.9 µg/dL).	Hon (2010, 2011); may overlap with Hon 2009
Effect	110 children age ≤17			
Effect	58 children 10 years old	Cross-sectional	Atopic dermatitis severity, eczema severity score, and children's dermatology life quality index were positively correlated with blood Pb (mean, 1.9 µg/dL; mean age, 10).	Hon (2009) *Also for IgE*
Effect	2,470 children aged 5-14 in Germany	Ecological	Odds ratio for eczema was elevated in children from a polluted area in Germany that also had higher Pb dustfall; no blood Pb data.	Heinrich (1999) *Also for IgE and sens.*

Epidemiological studies of low-level Pb exposure, immunoglobulin E (IgE), sensitization, and eczema are listed by decreasing cohort size and grouped together for overlapping study groups. Blood Pb levels up to 15 µg/dL were included so that effects at and around 10 µg/dL were not excluded from the evaluation.

10 µg/dL, additional studies demonstrate increased IgE at mean blood Pb levels in the range of 2 µg/dL. Higher serum IgE was correlated with higher blood Pb (mean, 1.9 µg/dL) in 110 children with eczema in which the age ranged from 1 month to 17 years for the participants recruited from a dermatology clinic in Hong Kong (Hon *et al.* 2009, Hon *et al.* 2010, Hon 2011). Karmaus *et al.* (2005) reported increased serum IgE in a study of 331 children 7-10 years of age in Germany at blood Pb levels >2.8 µg/dL, the median for the group. The studies by Lutz *et al.* (1999), Sun *et al.* (2003), and Karmaus *et al.* (2005) include adjustments for age and sex in their analyses of the relationship between blood Pb and IgE. These are important considerations because of the strong age- and sex-related effects on serum IgE: higher IgE levels are observed in boys, and increased IgE levels are expected with increasing age up to puberty, with slowly decreasing levels observed thereafter (Lindberg and Arroyave 1986, Blackwell *et al.* 2011).

There is some evidence that in children the association of IgE with blood Pb may exhibit a nonmonotonic (or bi-phasic) dose response: increased IgE levels are reported at the lower end of blood Pb levels, from 2 to 20 µg/dL, but decreasing serum IgE levels occur at higher blood Pb levels (i.e., >20 µg/dL). Nonmonotonic dose responses are thought to reflect multiple mechanisms of toxicant action that affect a given endpoint (including immune effects) differently at different doses (Welshons *et al.* 2003). For example, Narita *et al.* (2007) described stimulation of IgE-mediated release of allergic mediators from human mast cells with surface-bound IgE in response to toxicants, including Aroclor 1242; a nonmonotonic dose response was reported for IgE-mediated release of β-hexosaminidase, with lower doses resulting in activation of this key step in allergic reactions and higher doses having no effect. Blood Pb levels >20 µg/dL (23-42µg/dL determined graphically from Wagnerova *et al.* 1986) were associated with decreased IgE in a study of 11-year-old children in Czechoslovakia in which both the exposed and reference group had blood Pb levels >10 µg/dL (Wagnerova *et al.* 1986). In the Lutz *et al.* (1999) study described earlier, serum IgE differed significantly ($p<0.05$ Kruskal-Wallis) by blood Pb levels stratified by CDC blood Pb classification levels (I=<10, IIA=10-14, IIB=15-19, and III=20-44 µg/dL); however, IgE was not increased in the group with blood Pb levels >20 µg/dL (IgE=52, 74, 210, and 64 IU/mL at blood Pb <10, 10-14, 15-19, and 20-44 µg/dL, respectively). In a similar analysis of 318 children in Egypt under 8 years of age, serum IgE also differed significantly ($p=0.001$ Kruskal-Wallis) by blood Pb levels stratified by CDC blood Pb classification levels (IA=<5, IB=5-9, IIA=10-14, IIB=15-19, III=20-44, IV=and 45-69 µg/dL); however, the correlation between blood Pb and IgE was not significant ($p=0.12$) for the overall group, which had a mean blood Pb of 9.2 µg/dL (Hegazy *et al.* 2011). The reason for the lack of a significant correlation with blood Pb in the Hegazy *et al.* (2011) study is not clear, but it may relate to differential effects of Pb at low and high blood Pb levels.

Prospective studies are not available to examine the relationship between blood Pb and serum IgE at later time points in children. However, in a study of newborns in Paris, Annesi-Maesano *et al.* (2003) demonstrated an association between higher umbilical cord IgE and higher infant hair Pb ($p<0.001$). Maternal blood Pb and infant blood Pb were not correlated to umbilical cord IgE levels, although the authors report that the association between maternal blood Pb and umbilical cord IgE was at borderline significance ($p<0.1$) (Annesi-Maesano *et al.* 2003). As discussed in **Section 3.2**, hair Pb has been examined in a number of studies because collection

is easy and minimally invasive; however, an ATSDR expert panel concluded that widespread use is not recommended because of unresolved scientific issues in collection and analysis (ATSDR 2001).

As discussed above, elevated levels of total IgE are associated with allergic sensitization and allergic disease such as asthma (Beeh *et al.* 2000, Kotaniemi-Syrjanen *et al.* 2002, Ahmad Al Obaidi *et al.* 2008, Donohue *et al.* 2008). However, there is *limited* evidence that blood Pb levels <10 µg/dL are associated with increased incidence of allergic sensitization in children. This conclusion is based two lines of evidence: (1) a prospective study reporting a significant association between maternal or umbilical cord blood Pb <10 µg/dL and greater incidence of sensitization to common allergens in children, and (2) data supporting Pb-associated increases in serum IgE in children. In a prospective study of the children of 224 women in Poland recruited in the second trimester of pregnancy, allergic sensitization or atopy was determined by skin prick test to common allergens administered when the children were 5 years of age (Jedrychowski *et al.* 2011). Frequency of sensitization was significantly associated with maternal blood Pb (p=0.006; mean Pb, 1.6 µg/dL) and with umbilical cord blood Pb (p=0.001; mean Pb, 1.2 µg/dL), but not with current blood Pb levels in the 5-year-olds (p=0.43; mean Pb, 2.0 µg/dL). In an analysis of the relative risk, umbilical cord blood Pb in the Jedrychowski *et al.* (2011) study was associated with an increased relative risk (RR=2.28 (95% CI: 1.1, 4.6)) of atopy as indicated by at least one positive skin prick test. The 224 individuals included in the statistical analysis all had umbilical cord blood Pb levels <2.5 µg/dL, because the authors state that outliers above the 95th percentile were removed before analysis. The effect of the removal of individuals with higher blood Pb levels is unknown. No other studies were located that reported blood Pb and sensitization; however, an ecological study supports the association between higher Pb exposure and higher sensitization in children (Heinrich *et al.* 1999). The odds ratio for positive skin prick test (OR=1.38 (95% CI: 1.02, 1.86)) and increased specific IgE (OR=1.75 (95% CI: 1.31, 2.33)) to common allergens were also elevated in children from an area in Germany with higher Pb dustfall and Pb emissions. The study examined 2,470 children 5-14 years of age; it lacked blood Pb data but compared children from areas with high Pb dustfall to children in the reference group living in a low-Pb-dustfall area (Heinrich *et al.* 1999).

There are few studies of eczema or atopic dermatitis in children, and the evidence of an association with blood Pb is restricted to a single group of 110 children at a dermatology clinic, which may represent a sensitive subpopulation. In two overlapping studies of 110 patients with eczema from a Hong Kong pediatric dermatology clinic, blood Pb was significantly associated with atopic dermatitis severity, eczema severity score, children's dermatology life quality index, and eosinophil count (Hon *et al.* 2009, Hon *et al.* 2010, Hon 2011). The association between blood Pb and clinical diagnosis for severity among the eczema patients is supported by the objective measure of a Pb-related increase in eosinophil count (r=0.27; p=0.001). The Hon *et al.* (2009, 2010, 2011) studies report an association between blood Pb and multiple clinical parameters rating severity of symptoms of atopic dermatitis, but not the incidence of eczema. Blood Pb levels did not differ between 110 eczema patients and 41 patients at the dermatology clinic with other skin conditions that did not have eczema (p=0.160); however, the study did not have a nonatopic reference group. The Heinrich *et al.* (1999) ecological study described earlier

also reported an increased odds ratio for eczema (OR=1.52 (95% CI: 1.03, 2.24)) among children living in the area with higher Pb dustfall. However, a retrospective study of 1,768 children born in Boston between 1979 and 1981, which determined relative risk of eczema in childhood with umbilical cord blood Pb levels, did not find a difference between children with umbilical cord blood Pb levels above and below 10 µg/dL (Rabinowitz *et al.* 1990). This study differs from the two Hon *et al.* (2009, 2010) studies in the timing of the Pb exposure measurement and in the reporting of incidence rather than severity of eczema. The Rabinowitz *et al.* (1990) study compared umbilical cord blood Pb to incidence of eczema years later (exact timing not reported) and addressed incidence (present vs. not present) rather than severity of eczema.

Although four retrospective studies examined the potential relationship between Pb exposure and asthma in children, the results are primarily negative, and only one of the four studies reported an association between asthma and blood Pb. Blood Pb levels >10 µg/dL were associated with an increased odds ratio for doctor diagnosis of asthma (OR=7.5 (95% CI: 1.3, 42.9)) in a study of 356 children <13 years of age in the STELLAR database in Michigan (Pugh Smith and Nriagu 2011). The analysis in Pugh Smith *et al.* (2011) was thoroughly adjusted for risk factors associated with asthma or confounders related to Pb exposure, such as age, gender, and exposure to passive smoke, cats, dogs, cockroaches, and other factors known to contribute to asthma. A retrospective study of 4,634 children in managed care in Michigan did not find an association between blood Pb at 1-3 years of age and incidence of asthma based on insurance records or dispensed medication in an analysis adjusted for income, birth weight, and sex (Joseph *et al.* 2005). A retrospective study of 1,768 children born in Boston between 1979 and 1981 that determined relative risk of asthma in childhood with umbilical cord blood Pb levels did not find a difference between children with umbilical cord blood Pb levels above and below 10 µg/dL in an analysis that did not include adjustment for confounders (Rabinowitz *et al.* 1990). Myers *et al.* (2002) reported that the incidence of asthma based on medical records did not differ between 151 patients in Chicago with high blood Pb levels (≥25 µg/dL) and a reference group with blood Pb <5 µg/dL. The Myers *et al.* (2002) tested for effects of high blood levels and did not report any adjustments for confounders.

The data from Sun *et al.* (2003) in girls and Pizent *et al.* (2008) in women suggest that Pb exposure may have a stronger effect on IgE in females. The results also indicate that analyses of Pb and IgE should consider sex as a potential confounder, which is not unexpected because sex differences in serum IgE are apparent early in childhood (Blackwell *et al.* 2011, Hunninghake *et al.* 2011), and other toxicant-induced or exacerbated hypersensitivity reactions have a gender bias (Corsini and Kimber 2007). All of the studies of IgE adjust for age, except the Hon *et al.* (Hon *et al.* 2009, Hon *et al.* 2010, 2011) set of studies. Therefore, as noted above, only the Karmaus *et al.* (2005) study reports an association with blood Pb levels <10 µg/dL in an analyses that adjusted for age. This represents a limitation in the data set because there are well-established age-dependent changes in serum IgE: increased IgE levels are expected with increasing age up to puberty, and slowly decreasing levels are observed thereafter (Blackwell *et al.* 2011). Smoking or exposure to passive smoke has also been associated with IgE. The effects

of smoke exposure may be two-fold, as exposure may increase IgE directly or it may lead to increased exposure to Pb because of the Pb content in tobacco smoke. In a study of 318 children in Egypt under 8 years of age, Hegazy *et al.* (2011) reported a significant correlation ($r=0.133$; $p<0.05$) between blood Pb and parental tobacco smoking. The analysis of IgE in the Karmaus *et al.* (2005) study was particularly thorough in its consideration and adjustment for confounders, including gender, age, number of infections in the last 12 months, exposure to passive smoke, and exposure to other toxicants including DDE (a metabolite of DDT that is also associated with increased IgE).

In adults, the results are mixed for an association between blood Pb and IgE or sensitization-related endpoints, but there are only three relevant studies in adults with blood Pb levels <10 µg/dL: a study of 523 office workers in Korea (Min *et al.* 2008), a study of 216 office workers in Croatia (Pizent *et al.* 2008), and a study of 94 Italians without occupational exposure to Pb (Boscolo *et al.* 1999, Boscolo *et al.* 2000). There was no correlation between blood Pb levels and serum IgE in men or women in the Boscolo *et al.* (1999, 2000) publications. Serum IgE was correlated to blood Pb levels in the women office workers in the Pizent *et al.* (2008) study, but not in the men. The Pizent *et al.* (2008) study also examined functional endpoints (sensitization to allergens by positive skin prick test and nonspecific bronchial reactivity by histamine challenge) as well as the observational data on serum IgE. Although blood Pb (range, 0.56-7 µg/dL) was associated with increased IgE in women, there was no effect of blood Pb on sensitization or bronchial reactivity. In men, blood Pb (range, 1-7 µg/dL) was not associated with serum IgE, and blood Pb was associated with decreased hypersensitivity responses, including decrease in sensitization to allergens and decrease in nonspecific bronchial reactivity (Pizent *et al.* 2008). In contrast, Min *et al.* (2008) reported a significant association between higher blood Pb (mean, 3 µg/dL) and higher nonspecific bronchial reactivity (by methacholine bronco-provocation test) in both male and female office workers. Two additional studies of IgE in adults support an association with Pb at higher blood Pb levels. Higher serum IgE was correlated with higher blood Pb levels in two studies: a study of 47 Pb refinery workers in Osaka with mean blood Pb 50 µg/dL (Horiguchi *et al.* 1992) and a study of 606 Pb battery workers in Korea with mean blood Pb 23 µg/dL in which IgE was elevated in workers with blood Pb >30 µg/dL compared with workers with blood Pb <30 µg/dL (Heo *et al.* 2004). Collectively, only four studies report data on blood Pb and IgE in adults. The two high-exposure studies (mean blood Pb levels, 23 and 50 µg/dL) suggest there may be an association between IgE and blood Pb ≥30 µg/dL (Horiguchi *et al.* 1992, Heo *et al.* 2004); the two studies with blood Pb levels <10 µg/dL are negative for effects in men and report mixed results in women (Boscolo *et al.* 1999, Boscolo *et al.* 2000, Pizent *et al.* 2008). An increase in symptoms of asthma and rhinitis was also reported in male industrial workers in the United Arab Emirates with extremely high blood Pb levels (mean, 78 µg/dL) relative to referents that also had high blood Pb levels (20 µg/dL) (Bener *et al.* 2001). In adults with blood Pb levels <10 µg/dL, the data for asthma or respiratory symptoms are conflicting, with one study reporting increased bronchial responsiveness (Min *et al.* 2008) and one study reporting decreased bronchial responsiveness or no effect of Pb (Pizent *et al.* 2008). The data for sensitization with blood Pb are negative, with no effect in female office workers and reduced sensitization with increasing blood Pb in males, although data are from a single study (Pizent *et al.* 2008).

Summary of support for conclusions

Animal data support an increase in IgE in adult mice at high Pb levels (50 µg injected subcutaneously three times per week for 3 weeks) and associated with developmental Pb exposure at levels that include blood Pb <10 µg/dL (2-20µg/dL in mice and 40µg/dL in rats; seeU.S. EPA 2006 for recent reviews of the animal data, ATSDR 2007). Prenatal or postnatal exposure to Pb at low, environmentally relevant levels in mice have been associated with increased IgE at later time points. Plasma IgE levels were significantly increased in neonatal BALB/c mice with blood Pb levels from 2 to 20 µg/dL at 2 weeks of age in mice that were the offspring of mothers exposed to Pb in drinking water during gestation alone, during lactation alone, or during both periods (unexposed neonate blood Pb was approximately 3µg/dL and maternal blood Pb not reported Snyder *et al.* 2000). A number of studies in mice also support Pb-associated skewing of T-cell response toward a type 2 helper T-cell (Th-2) response, which is associated with allergy and increased IgE production, versus a type 1 helper T-cell (Th-1) response, which is associated with host resistance and delayed-type hypersensitivity (reviewed in Dietert and Piepenbrink 2006, U.S. EPA 2006). The human data supporting a Pb-associated increase in serum IgE in children are restricted to cross-sectional studies. The determination of causation from cross-sectional studies has the inherent limitation of making conclusions based on current blood Pb measurements and lacking information on cumulative Pb or Pb exposure at earlier time points. The demonstration that infant hair Pb in newborns was associated with umbilical cord IgE levels from the Annesi-Maesano *et al.* (2003) study suggests that Pb exposure at earlier time points is associated with IgE in children, but prospective studies are lacking. The most relevant time for measuring exposure relative to IgE may include both earlier time points relating to mechanisms of Th-2 skewing (Parronchi *et al.* 2000) or current blood Pb directly related to secretion of IgE because IgE in serum has a half-life of less than 2 days. Five cross-sectional studies report a correlation between blood Pb (mean levels from 2 to 14 µg/dL) and serum IgE in children up to 17 years of age. Elevated levels of serum IgE were reported in children with increasing blood Pb across multiple studies from different populations in analyses that adjusted or controlled for age, sex, and in some cases smoking and exposure to other contaminants known to effect serum IgE. However, only the Karmaus *et al.* (2005) study reports an association at blood Pb levels <10 µg/dL in analyses that adjusted for age; therefore, the NTP concluded there is *limited* evidence that blood Pb <10 µg/dL is associated with elevated serum IgE in children. The conclusion of *limited* evidence for increased hypersensitivity responses at blood Pb <10 µg/dL in children is supported by the evidence for Pb-related increases in IgE together with the Jedrychowski *et al.* (2011) prospective study on allergic sensitization (diagnosed by skin prick testing). There are some data supporting an association between blood Pb <10 µg/dL and eczema or asthma in children; however, the conclusion of *inadequate* evidence is because the eczema studies with blood Pb are from a single patient group and represent 110 total children, and the data supporting an association with asthma are restricted to a single study. For adults, there are only three studies of IgE or sensitization-related effects of people with blood Pb levels <10 µg/dL. There is *inadequate* evidence in adults to address the potential association between blood Pb <10 µg/dL and IgE, allergy, eczema, or asthma. The NTP's conclusions for *limited* evidence for increased serum IgE in children at blood Pb levels <10 µg/dL are in line with other agencies, although the conclusions of a consistent association from the 2006 EPA AQCD for Lead (U.S. EPA 2006) and

significant associations in ATSDR's Toxicological Profile for Lead (ATSDR 2007) suggest slightly stronger conclusions by these agencies that may reflect the data from studies with blood Pb levels above 10 µg/dL.

5.3.2 IgG, IgM, IgA, and Antibody Response

There is *inadequate* evidence that blood Pb levels <10 µg/dL are associated with serum levels of IgG, IgM, or IgA in the blood of children or adults (see Appendix B: Immune Effects). Studies that examined serum immunoglobulins found no evidence of a consistent change, either increase or decrease, associated with blood Pb below or above 10 µg/dL. There is *inadequate* evidence that blood Pb at any level is associated with changes to the IgM- or IgG-specific functional antibody response. Two studies were located that evaluated an antibody response in humans in conjunction with blood Pb levels, and both reported that there was no effect of blood Pb level on the antibody response. There was no effect of blood Pb levels on the anti-rubella IgG antibody titer to a previous antigen (Rubella vaccine) in 279 children in Missouri from 9 months to 6 years of age (mean not reported, 65% had blood Pb <10µg/dL Lutz *et al.* 1999), and there was no difference in tetanus toxoid-specific antibodies between children with very high blood Pb levels (45 µg/dL) and high blood Pb levels (23 µg/dL) (Reigart and Graber 1976). The few studies that examined serum immunoglobulin levels (other than serum IgE discussed in the previous section) in children or adults with blood Pb levels <10 µg/dL report inconsistent results. Serum levels of IgG were correlated (r=0.31; p=0.002) with blood Pb levels (mean, <2 µg/dL), but IgM was not related to blood Pb in a study of umbilical cord blood from 101 newborns in Quebec (Belles-Isles *et al.* 2002). There was no effect of blood Pb level (mean, 3 µg/dL) on serum IgG, IgM, or IgA in a study of 331 children 7-10 years of age in Germany (Karmaus *et al.* 2005). Serum IgG, IgM, and IgA were increased in children with blood Pb ≥15 µg/dL compared to children with Pb <5 µg/dL in children <3 years of age at mean blood Pb level of 7 µg/dL; immunoglobulin levels were not related to blood Pb in children >3 years of age or in adults (Sarasua *et al.* 2000). In contrast, serum IgG and IgM were decreased in children in China 3-6 years of age with blood Pb >10 µg/dL from a sample of 72 children 3-6 years of age in China with mean blood Pb of 9.5 µg/dL (Sun *et al.* 2003). Blood Pb was not correlated with serum IgA, IgM, or IgG in a study of atopic and nonallergic men in Italy without occupational exposure to Pb (mean Pb, 11 µg/dL; (n=34 Boscolo *et al.* 1999).

Summary of support for conclusions

Animal data are mixed for an effect of Pb on the antibody response and most studies do not report serum immunoglobulin levels (seeU.S. EPA 2006 for recent reviews of the animal data, ATSDR 2007). Studies in mice report no effect, suppression, or stimulation of the T dependent antibody response (plaque-forming cell (PFC) assay to sheep red blood cell challenge) at blood Pb levels of 25-130 µg/dL (Blakley and Archer 1981, Mudzinski *et al.* 1986), while suppression of the PFC response was observed in rats at 29 µg/dL (Luster *et al.* 1978). The conclusion of *inadequate* evidence that blood Pb levels <10 µg/dL are associated with serum levels of IgG, IgM or IgA or functional antibody response in children or adults is based on the inconsistent results for serum IgG, IgM, and IgA and the general lack of human studies on Pb and the antibody response. The 2006 EPA AQCD for Lead (U.S. EPA 2006) and the 2007 ATSDR

Toxicological Profile for Lead (ATSDR 2007) do not make strong conclusions on the antibody response or serum immunoglobulins; however, the NTP's conclusions for *inadequate* evidence for an association between blood Pb levels <10 µg/dL and serum IgG, IgM, IgA, or the antibody response are consistent with the evidence presented in these documents.

5.3.3 T lymphocytes (T-cells)

There is *inadequate* evidence that blood Pb levels <10 µg/dL are associated with altered T lymphocyte (T-cell) numbers or percentages in the blood of children or adults (see Appendix B: Immune Effects). The results of studies at lower blood Pb levels (<10 µg/dL) are inconsistent; however, there are a number of occupational studies that report decreased absolute numbers or percentages of T-cells or T-cell populations or subsets (particularly CD4+ T-helper cells), and some include a corresponding increase in B-cells at blood Pb <15 or <30 µg/dL. An association between higher blood Pb and higher numbers of naive T-cells (CD45RA+ or CD45RO-; cells not yet programmed for a specific immune response) is reported in several studies at low (<10 µg/dL) and high blood Pb levels.

The results of studies in children and adults with blood Pb levels <10 µg/dL are inconsistent for a potential relationship between blood Pb and T-cell numbers. In a study of 70 children 3-6 years of age in China, the percentage of T-cells was unchanged, the percentage of CD4+ T-cells was decreased, and the percentage of CD8+ T-cells was increased in children with blood Pb levels >10 µg/dL compared to children <10 µg/dL (Zhao *et al.* 2004, Li *et al.* 2005). In contrast, Sarasua *et al.* (2000) reported decreased percentage of T-cells, no change in CD4+ or CD8+ T-cells, and increased percentage of B-cells in children under 3 years of age at mean blood Pb level of 7 µg/dL. Lymphocyte populations were not related to blood Pb levels in children >3 years of age or in adults (Sarasua *et al.* 2000). Four additional studies in children reported no effect of blood Pb on lymphocyte populations at mean blood Pb levels ranging from <2 µg/dL to 9 µg/dL in 318 children <8 years of age in Egypt (Hegazy *et al.* 2011), in 279 children from 9 months to 6 years of age in Missouri (Lutz *et al.* 1999), in 331 children 7-10 years of age in Germany (Karmaus *et al.* 2005), or in 101 newborns in Quebec (Belles-Isles *et al.* 2002). In adults with blood Pb levels <10 µg/dL, the results of studies that examined the relationship between blood Pb and lymphocyte populations are mixed. Higher blood Pb was correlated with higher numbers of CD4+ T-cells in atopic and nonallergic men (n=34 Boscolo *et al.* 1999) and with higher numbers of CD8+ T-cells in nonallergic women, but not in atopics, in Italy without occupational exposure to Pb (mean Pb, 5-11 µg/dL; n=60)(n=60 Boscolo *et al.* 2000) . Increased blood Pb was also correlated with increased numbers of CD4/CD45RO- naive CD4 T-cells in both nonallergic men and women, but not in atopics (Boscolo *et al.* 1999, Boscolo *et al.* 2000). Naive CD4/CD45RA+ CD4 T-cells were also increased in three-wheel drivers in India (mean, Pb=7 µg/dL; n=26) compared to a reference group (mean Pb, 5 µg/dL; n=59), but CD4+ T-cells were decreased (Mishra *et al.* 2010).

For higher blood Pb levels (i.e., >15 µg/dL), a number of occupational studies report altered T- or B-cells concentrations. In general, the absolute numbers or percentages of T-cell subsets (particularly CD4+ T-helper cells) are decreased and there is a corresponding increase in B-cells. No clear and consistent cell group is related to Pb levels, although several studies have shown

an association between higher blood Pb and greater numbers of naive T-cells. The numbers and percentages of T-cells and CD4+ T-cells were decreased in firearms instructors in the United States (mean Pb, 15 (n=36) and 31 µg/dL (n=15)) (Fischbein *et al.* 1993). The number of CD4 T-cells was reduced in 25 Pb battery workers in Turkey with very high mean blood Pb levels of 75 µg/dL (Undeger *et al.* 1996, Basaran and Undeger 2000). In a separate study of Pb battery workers with very high mean blood Pb of 132 µg/dL (n=33 in India), Mishra *et al.* (2010) reported that the percentage of CD4+ T-cells were decreased and percentages of CD45RA+ (naive) T-cells were increased. In contrast, Pinkerton *et al*. (1998) reported no effect on CD4+ T-cells, that higher B-cell counts were associated with higher Pb exposure, and that lower CD4/CD45RA+ naive CD4 T-cell counts were correlated with higher cumulative Pb exposure in a study of U.S. Pb smelter workers (median Pb, 39 µg/dL; n=145). Several studies have reported that CD4+ T-cells were not related to blood Pb but found either decreases in CD8+ T-cells (Garcia-Leston *et al.* 2011) and decreased CD8+ T-cells along with decreased B-cells (Kuo *et al.* 2001), or increased percentages of CD8+ T-cells (Sata *et al.* 1998).

The issue of reverse causality is also possible because Pb in whole blood is largely contained in the circulating blood cells, although this is generally attributed to the red blood cells, not to the white blood cells or leukocytes (lymphocytes are a subpopulation of leukocytes). Choi and Kim (2005) reported that boys with higher blood Pb levels had higher counts for leukocytes, and these counts correlated significantly with blood Pb levels (mean, 3 µg/dL; $r=0.39$; $p<0.05$) in a study of 251 adolescents 13-15 years of age in South Korea; the authors did not report data for lymphocytes or for specific lymphocyte subsets (e.g., T-cells). Higher blood Pb levels in these individuals may cause higher leukocyte counts, or higher circulating leukocyte counts may lead to higher measurements of blood Pb if the leukocytes contain substantial amounts of Pb.

Summary of support for conclusions

Animal data support a Pb-associated effect on T-cell maturation, and particularly a shift toward the type 2 helper T-cell (Th-2) phenotype, which is associated with allergy and increased IgE production, versus a type 1 helper T-cell (Th-1) response, which is associated with host resistance and DTH response (reviewed in Dietert and Piepenbrink 2006, U.S. EPA 2006). Some animal data support Pb-associated decreases in T-cell populations (e.g., decreased thymic CD4 and CD8 T-cells with Pb exposure and Listeria infection in BALB/c mice at blood Pb <25µg/dL Dyatlov and Lawrence 2002); however, decreased T-cell populations with Pb exposure are not widely reported in the experimental animal literature. White blood cell differentials with enumeration of lymphocyte subsets (T-cells, B-cells, CD4 and CD8 T-cells) are among the most common immune assays used in human studies, in part because of the relative ease of the assay and ability to obtain data from a small blood sample. Values typically have a large degree of variation that is influenced by sex, race, age, and methodological differences in obtaining and processing the samples. It is worth noting that lymphocyte subset analysis does not evaluate immune function, although it is an accepted part of a tiered screening approach to the evaluation of potential immunotoxicity of a given chemical (Luster *et al.* 1992). Differential white blood cell counts are not particularly sensitive indicators of immunotoxicity, and statistically significant effects associated with exposure often fall within normal ranges for the population. Pb-associated changes in T-cell counts or percentages at lower exposure levels are

relatively small and may be without a functional impact on the immune response of individuals. However, on a population level, a small change in cell numbers or percentages may be adverse. A good example is the demonstration that increased mortality risk is associated with small decreases in CD4 and total white blood cell counts in a study of people ≥85 years of age (Izaks *et al.* 2003).

Although no clear or consistent cell population relates to Pb levels, changes in T-cell populations are reported in a wide range of human studies. Decreased T-cells or CD4 T-cells (particularly at blood Pb >15 μg/dL) and increased naive T-cells may be a biological signal of Pb exposure in humans. The NTP's conclusion of *inadequate* evidence that blood Pb levels <10 μg/dL are associated with altered T-cell abundance in the blood of children or adults is based on the lack of a clear pattern of results in studies with lower blood Pb levels. Changes in T-cell populations do not equate to a functional immune outcome, although they may be relevant to changes in the DTH response. Although a large body of data from animals demonstrates clear and consistent Pb-associated suppression of the DTH response in mice, rats, goats, and chickens (see discussion of the animal data on DTH below and Dietert and Piepenbrink 2006 for review, U.S. EPA 2006), no studies of Pb and the DTH response were located in humans. The EPA 2006 AQCD for Lead (U.S. EPA 2006) and 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) both include changes in T-cell subpopulations as characteristic immune effects identified with Pb exposure at higher levels (e.g., 30-70 μg/dL in ATSDR).

5.3.4 Monocyte/Macrophages

There is *inadequate* evidence that blood Pb levels <10 μg/dL are associated with changes in macrophage function. The human data on macrophage function are limited to a single study of 65 children 6-11 years of age in Mexico living near a Pb smelter (mean blood Pb levels of 21 and 30 μg/dL) compared to children with blood Pb levels of 7 μg/dL (Pineda-Zavaleta *et al.* 2004). The study investigated nitric oxide (NO) and increased superoxide (O_2^-) production by macrophages. At appropriate levels, both NO and O_2^- are involved in destruction of bacteria by macrophages and other cells. Decreased macrophage NO and increased O_2^- production after indirect (phytohemagglutinin) stimulation through lymphocytes as well as direct (interferon-γ/lipopolysaccharide) stimulation of the macrophages were observed in cell cultures from the Pb-exposed boys but not from Pb-exposed girls. Animal data provide strong support for decreased NO and increased O_2^- or reactive oxygen intermediate production by macrophages after in vivo or in vitro exposure to Pb but generally lack blood Pb data (see U.S. EPA 2006 for recent reviews of the animal data, ATSDR 2007). Production of the proinflammatory cytokine (tumor necrosis factor-α (TNF-α)) by macrophages is also associated with Pb exposure, and the animal data suggest that production of TNF-α by macrophages is linked to increased sensitivity to bacteria-derived endotoxin (see U.S. EPA 2006 for recent reviews of the animal data). In vitro studies of human macrophages and other mononuclear cells in blood demonstrated increased production of TNF-α with Pb exposure (Villanueva *et al.* 2000) in the presence of lipopolysaccharide plus Pb (Guo *et al.* 1996). However, low levels of Pb were associated with suppression of TNF-α release in human mononuclear cells from blood in the presence of heat-killed *Salmonella enteritidis* (Hemdan *et al.* 2005). The 2006 EPA AQCD for Lead (U.S. EPA 2006) identifies stimulation of a hyper-inflammatory state in macrophages as one of the principal

immune effects of Pb; however, it notes that there is a general lack of human epidemiological data in this area.

5.3.5 Neutrophils

There is *inadequate* evidence that blood Pb levels <10 µg/dL are associated with changes in neutrophil function. There are limited human data on the potential association between Pb exposure and neutrophils, and the studies are restricted to occupationally exposed individuals with mean blood Pb levels that are >30 µg/dL (see Appendix B: Immune Effects). Several studies report that movement of neutrophils toward their targets (chemotaxis) may be reduced in Pb workers at high blood Pb levels (Governa *et al.* 1988, Queiroz *et al.* 1993) or after in vitro exposure to Pb (Governa *et al.* 1987). There is also evidence that lytic activity of *Candida* may be reduced (Queiroz *et al.* 1994) but phagocytic activity is relatively unaffected in Pb workers (Guillard and Lauwerys 1989, Queiroz *et al.* 1994). There are few animal data on neutrophil function, and in humans there is a complete lack of data with lower blood Pb levels. Additional studies of neutrophil function are required to clarify the potential relationship to blood Pb in adults and children. The 2006 EPA AQCD for Lead (U.S. EPA 2006) and the 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) identify changes in neutrophil chemotaxis as a consistent finding with Pb exposure; however, the studies do not include blood Pb levels <10 µg/dL.

5.4 Susceptible Populations or Life Stages

Segments of the population that are may be more susceptible to health effects of Pb are discussed more extensively in **Section 3.0 Exposure**. A significant body of literature supports the developmental period as a susceptible window for immunotoxicity, with immune effects of developmental toxicant exposures occurring at lower doses and adverse effects may be more persistent than similar exposures in adults (Luebke *et al.* 2006, Dietert 2008). As described above, the database of immune studies of Pb in humans provides *limited* evidence that blood Pb levels <10 µg/dL are associated with increased hypersensitivity responses and allergic sensitization diagnosed by skin prick testing in children; however, there is *inadequate* evidence to assess these effects in adults. Children may represent a sensitive life stage for the effects of Pb on IgE and IgE-related effects, but there is not enough data on these endpoints in adults with low blood Pb levels to make this determination.

There are some established age-related differences in immunity including a general decline in IgE and allergic symptoms with increasing age that is more pronounced in the elderly (Mediaty and Neuber 2005). However, there is some evidence that the apparent age-related decrease in hypersensitivity may be associated with more than age-related decline, and the difference may reflect an increase in IgE and sensitization rates in recent cohorts (Jarvis *et al.* 2005). In either case, childhood and prenatal exposure periods are potentially susceptible life stages because of the elevated background level of IgE and IgE-mediated hypersensitivity. In addition, young children have higher levels of Pb exposure related to early hand-to-mouth behaviors. Therefore, children may experience higher levels of Pb during a life stage that is already characterized by elevated IgE.

There are also gender-related differences in immune function, with many studies reporting higher total IgE levels in boys and men than in girls or women (e.g., see Raby *et al.* 2007). A recent meta-analysis of over 550 studies reported that boys make up 64% of children age 18 or younger with allergies but that women make up 65% of adults above the age of 18 with allergies (Kelly and Gangur 2009). The Pb epidemiological data include two studies that report significant effect of gender on the effects of Pb on IgE. Sun *et al.* (2003) reported that increased IgE was statistically significantly correlated with blood Pb in girls in China with blood Pb ≥10 µg/dL 3-6 years of age but that the effect was not significant in boys. Similarly, Pizent *et al.* (2008) reported that serum IgE was correlated to blood Pb levels in female office workers in Croatia, but not in the males. These data suggest that females may be more susceptible to the effects of Pb on IgE and allergy. Alternatively, the higher basal IgE levels in males may make it more difficult to detect the effects of Pb.

The risk factors for hypersensitivity and allergies include age and sex discussed above, but evidence suggests that heredity is by far the most important factor (De Swert 1999). This may manifest as a difference by race; for example, Joseph *et al.* (2005) reported that African American children were at statistically significantly greater risk of asthma compared to Caucasians, regardless of blood Pb level. The general or background allergic status of mothers or of children may be relevant to the effects of Pb on IgE and hypersensitivity. Annesi-Maesano *et al.* (2003) reported that the association between infant hair Pb levels and umbilical cord blood IgE were affected by the allergic status of the mothers. In analyses dividing mothers by history of IgE-mediated allergic status (either allergic, as indicated by asthma, allergic rhinitis, or eczema, or nonallergic), the study reported that infant hair Pb was statistically significantly associated with increased IgE in children born to nonallergic mothers ($r=0.21$; $p<0.01$), but the relationship was not significant for children born to allergic mothers ($r=0.12$; $p>0.05$). The authors suggest that family history of allergy (in other words, atopy) may overshadow the effect of Pb on IgE in children. It is unclear from these data whether this reflects genetic factors predisposing the children to allergy (e.g., Raby *et al.* 2007, Hunninghake *et al.* 2008, Hunninghake *et al.* 2011) or whether prenatal exposure to cytokines, histamine, or other factors are important. In the one prospective study available, Jedrychowski *et al.* (2011) controlled for maternal allergic history (or atopy) in the analysis that demonstrated a statistically significant association between higher umbilical cord blood Pb and atopic status as indicated by a positive skin prick test to at least one common allergen in the children at 5 years of age. Furthermore, adjustment for maternal atopy, as well as child's gender, parity, maternal age, maternal education, and environmental tobacco, had very little effect on the relative risk (before adjustment: RR=2.20 (95% CI: 1.17, 4.16); after adjustment: R=2.28 (95% CI: 1.12, 4.62)). The association with maternal blood Pb also had a relatively small effect, but it did change the results from statistically significant to borderline (before adjustment: RR=1.81 (95% CI: 1.10, 3.00); after adjustment: R=1.72 (95% CI: 0.98, 3.00)) (Jedrychowski *et al.* 2011).

5.5 Pb Exposure Measurements

The following brief discussion outlines several Pb exposure issues that are directly relevant to immune effects of Pb. An expanded discussion is included in a separate section of this document (see **Section 3.0 Exposure**). No studies of immune effects in humans were located that used a measure of exposure other than blood Pb and hair Pb. However, it is important to note that blood Pb is only one measure of exposure, and it reflects only a portion of the Pb that is present in a given subject. The half-life of Pb in blood is approximately 35 days and therefore blood Pb is considered a good indicator of recent exposure. Most of the Pb is stored in bones (approximately 90% in adults, 80% in adolescents, and 66% in children under 5 years of age), and Pb from past environmental exposure is released into the blood, contributing to a chronic internal source of exposure (Leggett 1993).

The higher levels of Pb in bone may be particularly relevant for cells of the immune system and immune function. All of the white blood cells or leukocytes that develop postnatally are derived from progenitor cells in the bone marrow in a process termed *hematopoiesis*. Elevated Pb concentrations in bone are therefore of direct concern for the development of immune cells, and future studies should more closely consider the potential relationship between bone Pb levels and immune effects. Mechanistic studies in animals support the importance of Pb exposure on the development and differentiation of leukocytes. For example, Gao *et al.* (2007) reported that Pb modified bone-marrow-derived dendritic cells to promote Th-2 phenotype and immune responses associated with allergy and increased IgE production. The location of Pb within blood is also of particular relevance to immune function. The Pb in blood is mainly contained in cells, primarily in red blood cells, with less than 1% in serum. As discussed in **Section 5.3.3 T lymphocytes (T-cells)** above, Choi and Kim (2005) reported that blood Pb concentrations (mean, 3 µg/dL) were 2 fold higher in boys with higher leukocyte counts, and leukocyte counts correlated significantly with blood Pb levels ($r=0.39$; $p<0.05$), in a study of 251 adolescents 13-15 years of age in South Korea. The issue of reverse causality is suggested: higher blood Pb levels in these individuals may cause higher leukocyte counts, or higher circulating leukocyte counts may result in elevated blood Pb concentrations if the leukocytes contain substantial amounts of Pb in the boys in this study.

5.6 Delayed-type Hypersensitivity (DTH) and Pb-related Immune Effects in Animal Studies

There is a large body of laboratory animal data on immune effects of Pb. The effects in animal models support the human data, as discussed above for increased IgE, with evidence to support a Th-2-related mechanism and proinflammatory shift in macrophage function. However, the major effect on immune function associated with Pb exposure appears to be suppression of the delayed-type hypersensitivity (DTH) response. Animal data provide strong and consistent support for Pb-associated suppression of DTH response (see U.S. EPA 2006, ATSDR 2007 for recent reviews of the animal data). The DTH response depends on priming and expansion of antigen-specific T-cells that are Th-1 dependent and therefore the Pb-associated suppression of Th-1 responses is consistent with the Pb-associated suppression of DTH. The DTH response is a functional immune endpoint that is widely accepted as an indicator of cell-mediated function

(Luster *et al.* 1992, U.S. EPA 1998). Although DTH response can be evaluated as a measure of immune response in humans (e.g., Vukmanovic-Stejic *et al.* 2006), no studies were located that evaluate the relationship between blood Pb and DTH in humans. Studies on the DTH response in humans with low blood Pb levels are recommended because of the lack of data in humans and the clear and consistent Pb-related suppression of the DTH response in mice, rats, goats, and chickens.

Exposure to Pb is associated with suppression of the DTH response in mice, rats, goats, and chickens, and following both acute and subchronic exposures of up to 16 weeks. Although blood Pb levels are not available in all studies, decreased DTH response has been reported at blood Pb levels from 29 to 87 µg/dL. Wistar rats that were the offspring of dams exposed from premating to weaning, and continued to receive 25 or 50 ppm Pb acetate in drinking water until tested, had suppressed DTH at blood Pb level of 29 and 52 µg/dL (Faith *et al.* 1979). Adult BALB/c mice exposed to 32, 128, 512, or 2048 ppm Pb acetate in drinking water for 3 weeks had blood Pb levels of 9, 49, 87, and 169 µg/dL, and the blood Pb level correlated with suppressed DTH response (McCabe *et al.* 1999). Although McCabe *et al.* (1999) report that the DTH response in the mice with 87 µg/dL blood Pb level was statistically suppressed, it is not clear from the paper if blood Pb 9 µg/dL (a more environmentally relevant level) was associated with suppressed DTH response. In a study of maternal exposure of Fisher 344 rats to 250 ppm Pb acetate, maternal blood Pb levels were as high as 66 µg/dL; however, there was no effect of Pb exposure in the dams 8 weeks after parturition and the Pb exposure was stopped (Chen *et al.* 2004). In contrast, the offspring with blood Pb levels between 6 and 8 µg/dL measured 4 weeks after the dams were last exposed had significantly suppressed DTH response. The developmental nature of this study and the early removal of Pb exposure to the dams suggest that the DTH effect of Pb has a clear developmental component. No experimental animal data report a blood Pb level associated with a "no effect" level, and therefore the lower blood Pb range associated with effects is unknown, even in laboratory animals.

5.7 Conclusions

The NTP concludes that there is *limited* evidence that blood Pb levels <10 µg/dL are associated with adverse immune effects in children and *inadequate* evidence in adults (see **Table 5.4** for complete list of immune effects conclusions). In children, there is *limited* evidence that blood Pb levels <10 µg/dL are associated with increased hypersensitivity responses and allergic sensitization diagnosed by skin prick testing to common allergens. There is also *limited* evidence that blood Pb levels <10 µg/dL are associated with elevated serum IgE levels. The data supporting Pb-related increases in IgE, together with a prospective study on allergic sensitization, provide *limited* evidence that blood Pb <10 µg/dL is associated with increased hypersensitivity responses in children. In some studies blood Pb levels ≤2 µg/dL are associated with increased serum IgE (e.g., Karmaus *et al.* 2005, Hon *et al.* 2010) or increased sensitization to common allergens indicated by positive skin prick test (e.g., Jedrychowski *et al.* 2011). There is *inadequate* evidence of an association between blood Pb and eczema or asthma in children and *inadequate* evidence in adults to address the potential association between blood Pb <10 µg/dL and IgE, allergy, eczema, or asthma. There is *inadequate* evidence that blood Pb

levels <10 µg/dL are associated with observational data such as altered lymphocyte counts or serum levels of IgG, IgM, or IgA in the blood of children or adults because of a general lack of studies at the lower dose and inconsistency in available data. There is *inadequate* evidence that blood Pb levels <10 µg/dL are associated with changes in immune function other than hypersensitivity because there are few studies of Pb and immune function in humans, particularly at lower blood Pb levels. Very few studies examine markers of exposure other than blood Pb levels, and therefore it is unknown if blood or bone Pb levels would be a better measure of exposure for Pb-related immune effects.

Table 5.4: NTP conclusions on immune effects of low-level Pb

Health Effect	Population or Exposure Window	NTP Conclusion	Blood Pb Evidence	Bone Pb Evidence
Increased serum immunoglobulin E (IgE)	Prenatal	*Inadequate*	Unclear	Hair Pb data
	Children	*Limited*	Yes, <10 µg/dL	No data
	Adults	*Inadequate*	Unclear	No data
Increased hypersensitivity and allergy (e.g., positive skin prick test)	Prenatal	*Limited*	Maternal and umbilical cord <10 µg/dL	No data
	Children	*Limited*	Yes, <10 µg/dL	No data
	Adults	*Inadequate*	Unclear	No data
Asthma, eczema, etc.	Prenatal	*Inadequate*	Unclear	No data
	Children	*Inadequate*	Unclear	No data
	Adults	*Inadequate*	Unclear	No data
Altered serum IgG, IgM	Prenatal	*Inadequate*	No data	No data
	Children	*Inadequate*	Unclear	No data
	Adults	*Inadequate*	Unclear	No data
Altered antibody response	Prenatal	*Inadequate*	No data	No data
	Children	*Inadequate*	No data	No data
	Adults	*Inadequate*	No data	No data
Immunophenotyping (e.g., T-cells, B-cells)	Prenatal	*Inadequate*	No data	No data
	Children	*Inadequate*	Unclear	No data
	Adults	*Inadequate*	Unclear; >15 µg/dL data suggest changes in T-cells or T-cell subpopulations	No data
Monocyte/macrophage function	Prenatal	*Inadequate*	No data	No data
	Children	*Inadequate*	Unclear (one study)	No data
	Adults	*Inadequate*	No data	No data
Neutrophil function	Prenatal	*Inadequate*	No data	No data
	Children	*Inadequate*	No data	No data
	Adults	*Inadequate*	Unclear; >30 µg/dL data suggest changes in chemotaxis and lytic activity	No data
Delayed-type hypersensitivity (DTH) response	Prenatal	*Inadequate*	No data	No data
	Children	*Inadequate*	No data	No data
	Adults	*Inadequate*	No data	No data

6.0 CARDIOVASCULAR EFFECTS

6.1 Conclusions

NTP concludes that there is *sufficient* evidence that blood Pb levels <10 µg/dL in adults are associated with adverse effects on cardiovascular function.

There is *sufficient* evidence of a bone-Pb-related increase in blood pressure (BP) and the risk of hypertension. Two prospective studies and five cross-sectional studies found a statistically significant association between bone Pb and increased BP or hypertension. These studies were in populations or samples with blood Pb levels <10 µg/dL, and most of them had mean levels <5 µg/dL. Blood Pb was less consistently associated with BP and hypertension in adults. Studies of groups with mean blood Pb levels <5 µg/dL (often more recent studies) have found significant associations between concurrent blood Pb and higher BP. The NTP recognizes that an individual with blood levels <10 µg/dL during adulthood may have had higher blood Pb levels earlier in life, and the role of early-life Pb exposure cannot be discriminated from the role of concurrent blood Pb.

There is *sufficient* evidence that blood Pb levels <10 µg/dL are associated with an increased risk of hypertension during pregnancy. One prospective study and five cross-sectional studies supported an association, all with mean blood Pb levels <10 µg/dL. There is *limited* evidence of increased risk of cardiovascular-related mortality associated with blood Pb levels <10 µg/dL based on three prospective studies that report an association with blood Pb, and two prospective studies that did not support an association with blood Pb at mean levels of 11.5 µg/dL and 5.6 µg/dL. There is also *limited* evidence for Pb effects on ECG abnormalities and cardiovascular disease including cerebrovascular disease and peripheral arterial disease, because there are few replicated studies of blood Pb effects.

There is *inadequate* evidence to assess whether children present a sensitive life stage for cardiovascular effects of Pb. No prospective studies have followed children with early-life Pb measures with determination of cardiovascular health after childhood, and the few studies of blood Pb and BP during childhood had inconsistent results. There is some evidence that menopause is associated with higher blood Pb levels, associated with mobilization of bone stores of Pb, and this could put women at greater risk of Pb-related cardiovascular effects. However, there are few studies and *inadequate* evidence to assess cardiovascular effects of Pb in menopausal women. One cross-sectional study (mean blood Pb) found a stronger statistically significant association between blood Pb and hypertension in postmenopausal women (Nash *et al.* 2003), but two smaller studies found no association (Pizent *et al.* 2001, Al-Saleh *et al.* 2005). There is *inadequate* evidence for Pb effects on heart rate variability or specific types of cardiovascular disease; due to few replicated studies.

Chronic Pb exposure appears to be more critical than current Pb exposure, as indicated by more consistent associations with bone Pb than with blood Pb for chronic cardiovascular effects such as hypertension and mortality from cardiovascular causes. The data are inadequate to evaluate

prenatal or childhood Pb exposure with health effects at later stages of development or in adulthood.

Although the cardiovascular and renal systems are intimately linked, effects are considered separately in this evaluation because studies generally reported individual effects rather than testing both systems comprehensively. Nevertheless, it is recognized that kidney dysfunction can contribute to increased BP and that hypertension can contribute to adverse renal effects. The EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) both conclude that cardiovascular and renal effects of Pb may share biological mechanisms. As discussed in **Section 6.4,** multiple studies suggest that individuals with decreased renal function are a susceptible population for adverse cardiovascular effects of Pb.

6.2 How Conclusions Were Reached

Conclusions in the NTP evaluation of Pb-related cardiovascular effects in humans associated with low-level Pb were derived by evaluating the data from epidemiological studies with a focus on blood Pb levels <10 µg/dL. The NTP's conclusions are based on the evidence from human studies with blood Pb levels of <10 µg/dL, with data reflecting exposure levels up to 15 µg/dL. This section of the evaluation focuses primarily on the human data for cardiovascular effects of Pb because there is a relatively large database of human studies for these endpoints. Major endpoints considered as potential indicators of effects of Pb on cardiovascular functions are listed and briefly described in **Section 6.2.1**. This document is not a review of the cardiovascular system or toxicity, and the reader is directed to published reviews for additional background. Key data and principal studies considered in developing the NTP conclusions are discussed in detail in **Section 6.3 Evidence for Pb-related Effects on Cardiovascular Outcomes**. The discussion of each cardiovascular effect begins with a statement of the NTP conclusion of whether the specific effect is associated with a blood Pb level <10 µg/dL or <5 µg/dL and the age group in which it is identified (childhood or adulthood). Most studies are prospective or cross-sectional within a life stage (childhood or adulthood exposure and outcome) unless otherwise indicated. The discussion also highlights the extent to which experimental animal data support the association between Pb exposure and cardiovascular effects. Although the information necessary to support the NTP conclusions is presented in **Section 6.3**, the complete data set of human studies with data on cardiovascular endpoints from Pb-exposed groups is included in Appendix C: Cardiovascular Effects, and individual studies are abstracted for further reference. The NTP made extensive use of recent government evaluations of health effects of Pb in the current assessment, and the relevant conclusions of the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) are briefly described in **Section 6.2.2** below.

6.2.1 Principal Measures of Cardiovascular Effects

Table 6.1 lists a number of key cardiovascular endpoints commonly evaluated in epidemiological studies (as defined by the American Heart Association Cardiac Glossary (http://www.heart.org/HEARTORG/Conditions/HeartAttack/HeartAttackToolsResources/Cardiac-Glossary_UCM_303945_Article.jsp) or studies of pulse pressure and heart rate variability cited below). Blood pressure (BP) is the most widely measured cardiovascular effect in studies of Pb exposure and is evaluated as a continuous measure (range, in mmHg) or as categories (dichotomized as hypertension vs. no hypertension). High BP increases the risk of myocardial infarction and stroke, and BP control is one of the primary strategies to prevent the development of cardiovascular disease, with evidence of efficacy in patients without prior heart disease (Law *et al.* 2009). *Pulse pressure* is the difference between systolic and diastolic blood pressure (SBP and DBP), and increases reflect arterial stiffness, a critical cardiovascular risk factor (Lakatta and Levy 2003). Heart rate variability is an indicator of cardiac autonomic function, with decreased variability being associated with risk of heart disease and mortality, and with clinical relevance in relation to Pb exposure (Park *et al.* 2006).

Table 6.1: Major Pb-related cardiovascular outcomes/effects

Cardiovascular Effect	Description
Blood pressure (BP)	The force exerted by the heart against the walls of the arteries (measured in millimeters of mercury (mmHg)), with a maximum during the pumping phase of the heartbeat (systolic, SBP) and a minimum when the heart muscle relaxes between beats (diastolic, DBP).
Hypertension	Medical term for high blood pressure (currently, SBP ≥140 or DBP ≥90) compared to an optimal BP of <120/80 mmHg. BPs of 120-139/80-89 mmHg are considered prehypertension.
Pulse pressure	The difference between SBP and DBP; a marker of arterial stiffness.
Heart rate variability	Changes in the interval between heart beats. Decreased variability is a marker of abnormal autonomic nervous system functioning.
Electrocardiographic (ECG) conduction abnormalities	Changes in the typical pattern of electrical activity of the heart, including the P wave (atrial activity), QRS wave (ventricle activity), and T wave (return to resting state).
Peripheral artery disease	Narrowing of arteries carrying blood to the arms and legs, caused by atherosclerosis.
Coronary heart disease	Narrowing of the arteries that supply blood and oxygen to the heart muscle, caused by atherosclerosis, and which can result in a myocardial infarction (also called ischemic heart disease).
Myocardial infarction	Medical term for a heart attack: damage to heart muscle resulting from a blocked blood supply.
Stroke	Death or injury to brain cells when a blood clot blocks an artery in or leading to the brain (ischemic) or when a blood vessel ruptures (hemorrhagic).
Cardiovascular mortality	Death attributed to heart or circulatory causes.

American Heart Association Cardiac Glossary (http://www.heart.org/HEARTORG/Conditions/HeartAttack/HeartAttackToolsResources/Cardiac-Glossary_UCM_303945_Article.jsp)

Electrocardiographic (ECG) conduction abnormalities have also been investigated in relation to Pb exposure. Major clinical cardiovascular effects that have been associated with Pb exposure

include peripheral artery disease, coronary heart disease (ischemic heart disease), and stroke (cerebrovascular disease). Cardiovascular disease mortality has also been related to Pb exposure.

The data available to evaluate each of the major effects are discussed in separate subheadings under **Section 6.3 Evidence for Pb-related Effects on Cardiovascular Outcomes** below.

6.2.2 Principal Conclusions from the 2006 EPA and 2007 ATSDR Pb Documents

The EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) both concluded that epidemiological studies support a relationship between higher Pb exposure and decreases in cardiovascular health, including increased SBP and DBP, higher incidence of hypertension, and increased incidence of cardiovascular disease and cardiovascular-related mortality (see **Table 6.2** for principal conclusions; see the original documents for complete conclusions). The association between elevated blood Pb and increased BP (SBP and DBP) is supported by a large body of literature, including cross-sectional (e.g., NHANES), prospective cohort (e.g., Boston Normative Aging Study), and occupational studies, as well as several meta-analyses (Staessen *et al.* 1994, Schwartz 1995, Nawrot *et al.* 2002). The EPA (U.S. EPA 2006) and ATSDR (ATSDR 2007) both stated that the data support an increase of approximately 1.0 mmHg in SBP and 0.6 mmHg in DBP for every doubling of the blood Pb level. Both agencies concluded that cumulative past Pb exposure, reflected in bone Pb levels, may be as important as, if not a more important than, current exposure as indicated by blood Pb level in the contribution to Pb-related increases in BP. Every 10 µg/g increase in bone Pb was associated with an odds ratio for hypertension of 1.28 to 1.86 over a bone Pb range of <1.0 µg/g to 96 µg/g (U.S. EPA 2006). ATSDR also highlighted the potential mechanistic link between cardiovascular and renal effects of Pb.

Table 6.2: Main conclusions for cardiovascular effects in the 2006 EPA AQCD for Lead and the 2007 ATSDR Toxicological Profile for Lead

"Epidemiologic studies support the relationship between increased lead exposure and increased deleterious cardiovascular outcomes, including increased blood pressure and increased incidence of hypertension. … The evidence for an association of Pb with cardiovascular morbidity and mortality is limited but supportive." (U.S. EPA 2006, pg 6-271)
"Population studies suggest that there is a significant association between bone-lead levels and elevated blood pressure. Blood lead levels (PbBs) also have been associated with small elevations in blood pressure." (ATSDR 2007, pg 21)

The NTP considered the conclusions and data summaries from the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007). In general, the NTP accepted the conclusions and agreed that the data support them. Differences from the ATSDR and EPA documents are identified for specific endpoints. The focus of EPA's document did not clearly discriminate effects below and above 10 µg/dL. Most of the studies of the quantitative relationship between blood Pb and SBP report mean blood Pb levels <10 µg/dL (e.g., table 6-2 in U.S. EPA 2006). However, the conclusion on the increase in BP associated with a doubling of blood Pb did not specify whether this doubling occurred at blood Pb levels <10 µg/dL. Some studies considered by the EPA, particularly those conducted before 1990 or in occupational

settings, had more than 90% of their subjects with blood Pb >10 µg/dL (Kromhout *et al.* 1985, Lockett and Arbuckle 1987, Gartside 1988). Such studies were not considered for the NTP conclusions on Pb effects at levels <10 µg/dL.

6.3 Evidence for Pb-related Effects on Cardiovascular Outcomes

6.3.1 Blood Pressure (BP) and Hypertension

There is *sufficient* evidence that blood Pb levels <10 µg/dL are associated with increases in BP and risk of hypertension (see the Blood Pressure (BP) and Hypertension section of Appendix C: Cardiovascular Effects). BP is the most widely studied cardiovascular measure in studies of Pb, because it is routinely and easily measured. An association between higher Pb levels and higher BP is most consistent in studies of bone Pb (see **Table 6.3**). Women are at particular risk: there is *sufficient* evidence that blood Pb <10 µg/dL increases the risk of hypertension during pregnancy. Adults with concurrent blood Pb levels <10 µg/dL may have had higher Pb levels in the past, so the role of current blood Pb cannot be separated from an effect of early-life Pb exposure.

There is *inadequate* evidence for Pb effects on BP or hypertension in children. The size of the increase in BP that is detected is relatively small (1-2 mmHg). It is well established, however, that small increases in BP levels at the population level can have a substantial public health impact by increasing the risk of hypertension and incident cardiovascular disease (Whelton *et al.* 2002).

Despite some inconsistency across human studies, meta-analyses conclude that Pb exposure is associated with increased BP levels (Nawrot *et al.* 2002, Navas-Acien *et al.* 2008). Meta-analyses can account for the relative contributions of each study and multiple publications on overlapping data sets. Several recent meta-analyses support a small increase in BP from both blood (Nawrot *et al.* 2002) and bone Pb (Navas-Acien *et al.* 2008). Small but significantly increased risks of hypertension were found with tibia Pb (for a 10 µg/g increase: OR=1.04 (95% CI: 1.01, 1.07)), and nonsignificant increases for patella Pb (for a 10 µg/g increase: OR=1.04 (95% CI: 0.96, 1.12) and blood Pb (for a 5 µg/dL increase: OR=1.02 (95% CI: 0.93, 1.13) (Navas-Acien *et al.* 2008). Neither meta-analysis focused on low-level Pb exposure; for example, of the 10 studies included in the Navas-Acien *et al.* (2008) meta-analysis, two had mean blood Pb levels over 30 µg/dL, while the other eight were <10 µg/dL.

Bone Pb: Long-term exposure to Pb is often reflected in bone Pb levels, and several studies reported a significant association of BP with bone Pb but not with blood Pb (Cheng *et al.* 2001, Gerr *et al.* 2002, Rothenberg *et al.* 2002). However, bone Pb must be measured during specialized clinic visits and has not been as widely studied. **Table 6.3** summarizes the bone Pb literature for BP and hypertension listed by study type and decreasing study size, grouped together for overlapping or shared study groups. Most cross-sectional studies found an

Table 6.3: Studies of the association between bone Pb and blood pressure and hypertension used to develop conclusions				
Relevance to Conclusions	**Study Description**	**Study Design**	**Key Cardiovascular Findings**	**Reference**
Effect	496 former Pb workers, USA	Prospective	In men with past occupational Pb exposure (mean blood Pb, 4.6 µg/dL), blood and tibia Pb were associated with annual increases in SBP, but not DBP, over 3 years of follow-up.	Glenn (2003)
Normative Aging Study, USA				
Effect	474 men	Prospective	Bone Pb was correlated with increased SBP at baseline and an increased risk of hypertension 3-6 years later, while blood Pb was not associated.	Cheng (2001)
	619 men	Cross-sectional	A positive association between pulse pressure and bone Pb in a group of older men was modified by genetic variations in the *HFE* gene.	Zhang (2010)
	593 men	Cross-sectional	In a group of older men, tibia Pb was positively association with pulse pressure, but not blood Pb, which was positively correlated with DBP.	Perlstein (2007)
	590 men	Cross-sectional	Bone and blood Pb levels were higher in hypertensives (mean blood Pb, <7 µg/dL), and tibia Pb independently increased the risk of hypertension.	Hu (1996)
	471 men	Cross-sectional	The relationship between blood Pb, tibia Pb, or patella Pb and hypertension or BP may be modified by dietary calcium intake in a group of men with low Pb levels.	Elmarsafawy (2006)
No effect	750 older men (513 with hypertension)	Case-control	Bone Pb in the patella and tibia was not associated with risk of hypertension in these older men with low blood Pb levels (mean, 6.3 µg/dL).	Peters (2007)
Effect	667 third-trimester or postpartum women, USA	Cross-sectional	Calcaneus bone Pb measured postpartum was associated with increased BP and hypertension during the third trimester (mean blood Pb, 2.3 µg/dL postpartum).	Rothenberg (2002)
Effect	964 adults, Baltimore Memory Study, USA	Cross-sectional	In these older adults, blood Pb (mean, 3.5 µg/dL) was associated with BP, while bone Pb was associated with risk of hypertension.	Martin (2006)
Effect	543 former Pb workers, USA	Cross-sectional	In men with prior occupational exposure, blood Pb was associated with increased BP and an increased risk of hypertension, but not tibia or DMSA chelatable Pb.	Schwartz (2000)
Effect	508 young adults, half lived near Pb as children, USA	Cross-sectional	In young adults, some with childhood Pb exposure, SBP and DBP were significantly increased in those with the highest bone Pb levels (>10 µg/g, blood Pb 3.15 µg/dL).	Gerr (2002)
Effect	284 women (89 with hypertension), Nurses' Health Study	Case-control	Patella Pb was associated with an increased risk of hypertension in these middle-age women without occupational exposures (mean, 3 µg/dL), but tibia and blood Pb were not significantly associated.	Korrick (1999)

Epidemiological studies of bone Pb exposure and blood pressure and hypertension are listed by study type and decreasing study size, grouped together for overlapping or shared study groups.

Abbreviations: BP, blood pressure; DBP, diastolic blood pressure; DMSA, dimercaptosuccinic acid, used in the treatment of Pb poisoning; SBP, systolic blood pressure.

association with bone Pb and hypertension in the general population (Hu *et al.* 1996, Rothenberg *et al.* 2002, Elmarsafawy *et al.* 2006, Martin *et al.* 2006). One cross-sectional study did not find bone Pb to significantly increase the risk of hypertension, while blood Pb was significantly associated (Schwartz and Stewart 2000).

Blood Pb: Studies of blood Pb and BP do not consistently support an association as compared to studies of bone Pb and BP (see Blood Pressure (BP) and Hypertension section of Appendix C: Cardiovascular Effects). One prospective study supports a modest increase in SBP with both blood and bone Pb (Glenn *et al.* 2003), while three publications from two prospective studies failed to show an association with SBP or DBP (Grandjean *et al.* 1989, Moller and Kristensen 1992, Staessen *et al.* 1996). The prospective studies that did not find a significant association were in groups with higher mean blood Pb levels (between 10 and 15 µg/dL at baseline in Møller and Kristensen (1992) and Staessen *et al.* (1996)) than in the supportive study (4.6 µg/dL in Glenn *et al*. (2003)). The Glostrup Population Study, which did not support an association, was also larger than the supportive cohort (1,052 subjects in Møller and Kristensen (1992) vs. 496 in Glenn *et al*. (2003)). Thus, the studies that do not support an association between blood Pb levels and increased BP are not necessarily underpowered or include less exposed groups compared to the supportive studies. Twenty-nine publications of cross-sectional analyses with mean blood Pb levels <15 µg/dL support a small increase in SBP or DBP, while 17 did not support a relationship (some studies had multiple publications, so not all results are independent; see Blood Pressure (BP) and Hypertension section of Appendix C: Cardiovascular Effects for a complete list of studies considered). Analysis of NHANES 1999-2006 restricted to subjects with blood Pb <10 µg/dL (n=16,222) found a significant association with increased SBP and DBP (Scinicariello *et al.* 2011).

Three prospective studies failed to find an association between blood Pb and hypertension (Grandjean *et al.* 1989, Staessen *et al.* 1996, Cheng *et al.* 2001), although one of them did find an association with bone Pb (Cheng *et al.* 2001). Blood Pb was associated with increased prevalence of hypertension in 10 cross-sectional studies, but one study found no association (Kim *et al.* 2008). Black men had a significantly increased risk of hypertension (adjusted prevalence OR=2.69 (95% CI: 1.08, 6.72) for 90th (≥3.50 µg/dL) vs. 10th (≤0.70 µg/dL) percentile) when restricted to 1,767 subjects with blood Pb <10 µg/dL from NHANES 1999-2006 (Scinicariello *et al.* 2011). Case-control studies of hypertension were also inconsistent: supporting blood Pb (Bakhtiarian *et al.* 2006), not supporting blood Pb while supporting bone Pb (Korrick *et al.* 1999), and not supporting either blood Pb (Al-Saleh *et al.* 2005) or bone Pb (Peters *et al.* 2007).

Pulse Pressure: Higher pulse pressure (the difference between SBP and DBP) is a marker of arterial stiffness, and there was no association with blood Pb in the Normative Aging, but tibia Pb above the median was associated with an increase of 4 mmHg in pulse pressure (Perlstein *et al.* 2007, Zhang *et al.* 2010). In Mexican-American male NHANES subjects with blood Pb <10 µg/dL (n=1925), there was a significant 1.4 mmHg increase in pulse pressure per unit increase in the natural log of blood Pb (Scinicariello *et al.* 2011).

Differential Impacts: Blood Pb and bone Pb may reflect variable cardiovascular effects of Pb with acute effects on transient measures, such as BP, and chronic effects on clinical disease, such as hypertension—the more permanent state of elevated BP (Navas-Acien *et al.* 2008). Martin *et al.* (2006) proposed this hypothesis when they reported a significant association between blood Pb and BP as well as between bone Pb and hypertension. A prediction model for bone Pb based on the Normative Aging Study was developed by Park *et al.* (2009b). When it was applied to data from NHANES III, they found relatively more significant associations between estimated bone Pb and hypertension (Park *et al.* 2009b). This data should be considered cautiously because it was developed with a new method for modeling exposure. However, it should be noted that the model-building Normative Aging Study population only included older men, and factors such as age, menopause, and past pregnancies are associated with the mobilization of bone Pb (Symanski and Hertz-Picciotto 1995).

Modifiers: Blood pressure itself is a transient measure influenced by important cofactors that may modify an association with Pb (see **Section 6.4 Susceptible Populations and Modifiers of Pb Exposure**). The increase in BP that is supported by the data is 1-2 mmHg per doubling of blood Pb, and on a population basis the impact is likely to be larger in susceptible populations such as pregnant women or individuals with a particular metabolic gene variant.

Pregnancy puts women at greater risk of hypertension, which can contribute to preeclampsia and other complications. The mobilization of bone Pb during pregnancy (Gulson *et al.* 1997, Rothenberg *et al.* 2000) (see further discussion in **Section 3.4 Modifiers of Pb Exposure**) may contribute to the more consistent association of Pb exposure with hypertension and the greater magnitude of the change in BP during pregnancy (see **Table 6.4**). The change (increase) in blood Pb levels during pregnancy was associated with hypertension during pregnancy in a prospective study (Sowers *et al.* 2002); similarly, maternal blood Pb levels during pregnancy were associated with hypertension in cross-sectional studies (Rabinowitz *et al.* 1987, Yazbeck *et al.* 2009) and a case-control study (Vigeh *et al.* 2004). One study did not find an association between umbilical cord blood Pb and hypertension in the mother but did find a significant association with maternal SBP and DBP before delivery (Wells *et al.* 2011). All of these studies had mean blood Pb levels <10 µg/dL, and two of the supportive studies were had mean Pb levels <2 µg/dL (Sowers *et al.* 2002, Yazbeck *et al.* 2009). Additional studies in pregnant women support an association between higher blood Pb and higher BP (Rothenberg *et al.* 1999, Magri *et al.* 2003) and between higher bone Pb and higher BP (Rothenberg *et al.* 2002).

Women experiencing menopause may be at an increased risk due to mobilization of bone Pb stores that may be associated with periods of demineralization; increased blood Pb levels have been demonstrated in postmenopausal women in several studies (e.g., Silbergeld *et al.* 1988, Symanski and Hertz-Picciotto 1995, Webber *et al.* 1995, Korrick *et al.* 2002). In NHANES III, among women 40-59 years of age untreated for hypertension, the association between blood Pb and hypertension was stronger in postmenopausal women, with statistically significant odds ratios for systolic hypertension (quartile 2 (blood Pb 2.1-3.0 µg/dL): OR=3.0 (95% CI: 1.3, 6.9) quartile 3 (blood Pb 3.1-4.6 µg/dL): OR=2.7 (95% CI 1.2-6.2) compared to quartile 1 (blood Pb 0.5-2.0 µg/dL) (Nash *et al.* 2003)). The odds ratios for systolic hypertension in premenopausal

Table 6.4: Studies of Pb and blood pressure and hypertension during pregnancy used to develop conclusions				
Relevance to Conclusions	**Study Description**	**Study Design**	**Key Cardiovascular Findings**	**Reference**
Effect	705 pregnant women, USA	Prospective	Change in blood Pb concentration during pregnancy (mean, 1.2 µg/dL) was associated with hypertension in pregnancy, including the serious complications of preeclampsia and toxemia.	Sowers (2002)
Effect	3,851 women at birth of child, USA	Cross-sectional	There was an increase in women's BP during labor with increased umbilical cord blood Pb, as well as an increased risk of pregnancy hypertension from low Pb levels (6.3 µg/dL), but not preeclampsia.	Rabinowitz (1987)
Pregnant women, Los Angeles, USA				
Effect	1,627 pregnant women	Cross-sectional	In the third trimester of pregnancy, increased blood Pb was associated with increased BP only in immigrant women, primarily Latina (mean blood Pb, 2.3 µg/dL).	Rothenberg (1999)
	667 third trimester or postpartum women	Cross-sectional	Postpartum calcaneus bone Pb was associated with increased BP and hypertension during the third trimester (mean blood Pb, 2.3 µg/dL postpartum).	Rothenberg (2002)
Effect	971 pregnant women, EDEN Study, France	Cross-sectional	Blood Pb levels in the second trimester (mean, 1.9 µg/dL) were correlated with BP before and after 36 weeks gestation and increased the risk of pregnancy induced hypertension, particularly in parous women.	Yazbeck (2009)
Effect	285 pregnant women, Baltimore THREE Study, USA	Cross-sectional	Umbilical cord blood Pb of the child was associated with increased BP in the mother during labor and delivery (Q4 ≥ 0.96 µg/dL vs. Q1 ≤ 0.46 µg/dL), but not with other BP-related pregnancy conditions.	Wells (2011)
Effect	143 third trimester primigravid women, Malta	Cross-sectional	Blood Pb during the third trimester was higher in pregnant women with hypertension during pregnancy (mean, 9.6 vs. 5.8 µg/dL) and correlated with SBP and DBP in all pregnant women.	Magri (2003)
Effect	110 pregnant women, half with gestational hypertension, Iran	Case-control	Blood Pb at delivery was higher in women with hypertension during pregnancy (5.7 vs. 4.8 µg/dL), but Pb levels did not correlate with BP in the hypertensive women.	Vigeh (2004)

Epidemiological studies of Pb exposure and blood pressure and hypertension during pregnancy are listed by study type and decreasing size, grouped together for overlapping or shared study groups.

Abbreviations: BP, blood pressure; DBP, diastolic blood pressure; EDEN, Etude des Déterminants pré et post natals du développement et de la santé de Enfant; SBP, systolic blood pressure; Q1, first quartile; Q4, fourth quartile; THREE, Tracking Health Responses to Environmental Exposures.

women were around 1.5 and were not statistically significant. Other, smaller studies found no association between blood Pb and BP or hypertension in postmenopausal women (Pizent *et al.* 2001, Al-Saleh *et al.* 2005).

Children are at greater risk of Pb exposure due to early hand-to-mouth behaviors (see further discussion in **Section 3.0 Exposure**). Few studies evaluating the effects of Pb on BP have been conducted in children (see **Table 6.5**). Young adults with childhood Pb exposure had higher bone Pb and 3-4 mmHg higher SBP and DBP (>10 µg/d vs. <1 µg/g, $p<0.05$) (Gerr *et al.* 2002). In the Oswego Children's Study, umbilical cord blood Pb levels were associated with BP at 9.5 years of age, and early-childhood blood Pb (mean age, 2.6 years) was associated with increased BP in response to acute stress tasks at 9.5 years of age—particularly in children with low socioeconomic status (Gump *et al.* 2005, Gump *et al.* 2007). Other studies of blood Pb in children did not find an effect on BP (Factor-Litvak *et al.* 1996, Factor-Litvak *et al.* 1999, Chen *et al.* 2006). The adult origin of disease from childhood Pb exposure has not been sufficiently studied to inform a conclusion on cardiovascular risks from early or chronic Pb exposure.

While most of the literature supports a role for Pb in risk of hypertension, the definition of hypertension is not consistent across studies. The current standard is ≥140 mmHg SBP and/or ≥90 mmHg DBP, but several studies used a higher (Grandjean *et al.* 1989, Apostoli *et al.* 1990, Bakhtiarian *et al.* 2006, Elmarsafawy *et al.* 2006), lower (Al-Saleh *et al.* 2005), or "borderline" (140-159 mmHg SBP and/or 91-94 mmHg DBP) (Staessen *et al.* 1996) definition of hypertension. Studies also differed by how subjects taking anti-hypertensive medication were included, with one study excluding these subjects from analyses of BP (Scinicariello *et al.* 2010) and several considering subjects hypertensive based only on medication use (Hu *et al.* 1996, Staessen *et al.* 1996, Nash *et al.* 2003, Al-Saleh *et al.* 2005, Muntner *et al.* 2005, Martin *et al.* 2006, Scinicariello *et al.* 2010).

Summary of support for conclusions on BP and hypertension

Animal studies provide strong evidence for low-level Pb elevating BP in humans and contributing to the onset of hypertension, even after the exposure to Pb has stopped (U.S. EPA 2006 pg 5-103, ATSDR 2007 pg 28). In rats, blood Pb levels as low as 2.15 µg/dL showed significant increases in both SBP and DBP compared to unexposed rats, while exposures resulting in blood Pb levels >29 µg/dL did not show further increases in BP (Tsao *et al.* 2000). Many in vivo and in vitro studies support oxidative stress as the mechanism by which Pb contributes to the pathogenesis of hypertension (see U.S. EPA 2006 for further review of animal and mechanistic studies). Experimental animals do not show consistent dose-dependent increases in risk of hypertension: low, but not high, levels cause hypertension in some models (U.S. EPA 2006 pg 5-124). This nonmonotonic (or bi-phasic) relationship could partially explain inconsistency observed in human studies, where studies with mean blood Pb levels <5 µg/dL were generally more supportive of a relationship with BP and hypertension (see Blood Pressure (BP) and Hypertension section of Appendix C: Cardiovascular Effects).

Table 6.5: Studies of childhood Pb exposure and BP				
Relevance to Conclusions	**Study Description**	**Study Design**	**Key Cardiovascular Findings**	**Reference**
Oswego Children's Study, USA				
Effect	122 children	Prospective	Cord blood Pb levels (mean, 3 µg/dL) were associated with increased SBP at 9.5 years old, while childhood Pb levels (mean, 4.6 µg/dL at 2.6 years old) were associated with an increased DBP, decreased stroke volume, and increased total peripheral resistance response to acute stress.	Gump (2005)
	122 children	Prospective	Family socioeconomic status interacted with blood Pb to increase BP and may interact with blood Pb to increase total peripheral resistance response to acute stress tasks in these children with low blood Pb (mean, 4.6 µg/dL at 2.6 years old).	Gump (2007)
	140 children, 9-11 years old	Cross-sectional	Concurrent blood Pb (median, 0.94 µg/dL) was not associated with BP or BP responses to acute stress in these children, but blood Pb was associated with measures of impaired cardiac function in response to acute stress tasks.	Gump (2011)
No effect	780 children with moderately high Pb, half treated with succimer, USA	Cross-sectional	In these children with Pb exposure (20-44 µg/dL), there was no association between blood Pb and BP, including after 5 years of follow-up when mean blood levels were 8 µg/dL. Chelation of Pb with succimer had no effect on BP.	Chen (2006)
Effect	508 young adults, half lived near Pb as children, USA	Cross-sectional	In young adults, some of whom had childhood Pb exposure, SBP and DBP was significantly increased in those with the highest bone Pb levels (>10 µg/g, blood Pb 3.15 µg/dL).	Gerr (2002)
No effect	144 children in the town unexposed to Pb; Pristina, Kosovo	Cross-sectional	For the children residing in the town without Pb exposure, the small positive correlation of blood Pb with BP was not statistically significant.	Factor-Litvak (1996)

Epidemiological studies of childhood Pb exposure and blood pressure and hypertension are listed by study type and decreasing size, grouped together for overlapping or shared study groups.
Abbreviations: BP, blood pressure; DBP, diastolic blood pressure; SBP, systolic blood pressure.

The conclusion of *sufficient* evidence for a Pb-related increase in BP and risk of hypertension is based on a large body of literature in humans, with significant associations that are more consistently found with bone Pb as a measure of exposure than with blood Pb. There is a small but *sufficient* literature supporting Pb-related increases in hypertension during pregnancy, while there is *inadequate* evidence for Pb effects on BP or hypertension in children. The NTP's conclusions for *sufficient* evidence for BP and hypertension at blood Pb levels <10 µg/dL expand the conclusions of EPA's 2006 AQCD for Pb (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007).

6.3.2 Heart Rate Variability

There is *inadequate* evidence to evaluate a potential association between Pb exposure and heart rate variability (HRV). HRV reflects sympathetic (low frequency only) and parasympathetic (high and low frequency) autonomic nervous system function, with decreases in variability indicating abnormal autonomic function (Park *et al.* 2006). The literature contained only four publications of Pb and HRV with mean blood Pb levels <10 µg/dL, and the results were not consistent (Jhun *et al.* 2005, Park *et al.* 2006, Park *et al.* 2008, Gump *et al.* 2011). In the Oswego Children's Study, concurrent blood Pb in children 9-11 years of age (median blood Pb, 0.94 µg/dL) was significantly associated with impaired autonomic response to acute stress tasks as evaluated by HRV (Gump *et al.* 2011). In adults there was an indication that Pb may modify the effect of other metals (Jhun *et al.* 2005) or air pollution (Park *et al.* 2008) on HRV. The EPA's 2006 AQCD for Lead (U.S. EPA 2006) mentions HRV as a possible intermediary between Pb exposure and cardiovascular mortality, and HRV is not considered in the ATSDR's Toxicological Profile for Lead (ATSDR 2007).

6.3.3 Electrocardiogram Abnormalities

There is *limited* evidence for Pb effects on electrocardiogram (ECG) abnormalities. The Normative Aging Study supports a role for bone Pb and ECG abnormalities at the time of Pb measurement and 8 years later for QT and JT/ST prolongation and intraventricular or atrioventricular conduction defects (Cheng *et al.* 1998, Eum *et al.* 2011). Polymorphisms in iron metabolism genes (*HFE*, *TFC2*, *HMEX-1*) may modify these relationships (Park *et al.* 2009a). The Oswego Children's Study found that early-childhood blood Pb and concurrent blood Pb were associated with decreased stroke volume and increased total peripheral resistance in response to acute stress tasks at 9.5 years of age, especially in subjects with lower socioeconomic status who had higher blood Pb (overall mean, 4.6 µg/dL at age 2.6) (Gump *et al.* 2005, Gump *et al.* 2007, Gump *et al.* 2011). The studies of ECG abnormalities by Cheng *et al.* (1998) and Gump *et al.* (2005) were included in the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and the ATSDR's Toxicological Profile for Lead (ATSDR 2007), but no conclusions were reached.

6.3.4 Clinical Cardiovascular Disease

There is *limited* evidence that blood Pb levels <5 µg/dL are associated with clinical cardiovascular disease (see **Table 6.6** and Clinical Cardiovascular Disease section of Appendix C:

Table 6.6: Studies of Pb and clinical cardiovascular disease used to develop conclusions				
Relevance to Conclusions	**Study Description**	**Study Design**	**Key Cardiovascular Findings**	**Reference**
No effect	1,052 adults >40 years old, Glostrup Population Study, Denmark	Prospective	After 14 years of follow-up, blood Pb levels dropped, but there was no association with **coronary heart disease** or **cardiovascular disease** (fatal and nonfatal cases) in adults with 11.5 µg/dL mean blood Pb at baseline 40 years of age in 1976.	Møller (1992)
Effect	837 men, NAS, USA	Prospective	Blood and patella bone Pb were associated with an increased risk of **coronary heart disease** later in life in these older men with low blood Pb levels (mean, 6.3 µg/dL).	Jain (2007)
No effect	13,043 Pb workers, SHSP, South Korea	Cross-sectional	Pb workers showed no increased risk of **coronary heart disease** or **cerebral vascular disease** at low levels of exposure (5-10 µg/dL vs. <5 µg/dL).	Kim (2008)
NHANES (≥ 1999)				
Effect	9,961 adults, 1999-2002	Cross-sectional	**Peripheral artery disease** prevalence and risk increased with increasing quartiles of blood Pb (highest ≥2.47 µg/dL) in these adults from the general population.	Muntner (2005)
	4,447 adults >40 years old, 1999-2002	Cross-sectional	Blood Pb was associated with **peripheral artery disease** in these older adults with low levels (mean, 1.95 µg/dL) after adjustment, including renal function.	Guallar (2006)
	2,125 adults >40 years old, 1999-2000	Cross-sectional	These older subjects with **peripheral artery disease** had higher blood Pb, the risk of **peripheral artery disease** increased with increasing Pb (highest quartile: >2.9 µg/dL).	Navas-Acien (2004)
Effect	420 male bus drivers, Bangkok, Thailand	Cross-sectional	**Aging index of second derivative finger photoplethysmogram waveform** (SDPTG-AI), an assessment of arterial properties, is correlated with blood Pb in male bus drivers (mean, 6.3 µg/dL) and may be an independent cardiovascular risk factor.	Kaewboonchoo (2010)
Effect	197 women, ARFYS, Austria	Cross-sectional	**Intima-media thickness of the common and carotid arteries** was increased at very low levels of Pb (highest tertile = >0.82 µg/dL).	Zeller (2010)
Effect	128 ceramic painters, Japan	Cross-sectional	Increases in blood Pb were associated with decreases in postural changes in **finger blood flow volume** consistent with an atherosclerotic effect, although most of the subjects had Pb levels >10 µg/dL and other cardiac function tests were not associated.	Ishida (1996)
Effect	130 myocardial infarction patients, 61 controls, Pakistan	Case-control	Patients admitted for **myocardial infarction** had higher hair Pb levels, increasing for second and third **myocardial infarction**, and survival of third **myocardial infarction** decreased with higher hair Pb.	Afridi (2010)

Epidemiological studies of Pb exposure and cardiovascular morbidities are listed by study type and decreasing size, grouped together for overlapping or shared study groups.

Abbreviations: ARFYFS, Athero-sclerosis Risk Factors in Young Females Study NAS, Normative Aging Study (Boston area, began in 1963 by the Veterans Administration); NHANES, National Health and Nutrition Examination Survey; SHSP, Special Health Surveillance Program.

Cardiovascular Effects). A positive association has been reported between blood Pb level and several related cardiovascular diseases, including peripheral arterial disease and coronary artery disease, as well as blood flow measures indicative of atherosclerotic vascular resistance.

For cardiovascular disease in general, particularly conditions exacerbated by increases in BP, there was an increased risk from Pb exposure. In large studies that found a relationship between Pb and BP, Pb was also associated with an increased incidence of coronary artery disease (Normative Aging Study: (Jain *et al.* 2007)) and prevalence of peripheral artery disease (NHANES: (Navas-Acien *et al.* 2004, Muntner *et al.* 2005, Guallar *et al.* 2006)), while in the Glostrup Population Study there was no association between Pb and BP or cardiovascular disease (Moller and Kristensen 1992).

In prospective analyses, the Normative Aging Study reported an increased risk of coronary heart disease (myocardial infarction or angina) with blood Pb (log blood Pb adjusted HR=1.45 (95% CI: 1.01, 2.06), p=0.05) and bone Pb (log patella Pb, adjusted HR=2.64 (95% CI: 1.09, 6.37), p=0.05) (Jain *et al.* 2007). The prospective Glostrup Population Study in Denmark with a mean blood Pb at baseline of 11.6 µg/dL failed to find an increased risk of coronary heart disease or cardiovascular disease (Moller and Kristensen 1992).

A large cross-sectional study in Korea with low blood Pb (geometric mean, 6 µg/dL) found no increased risk for coronary heart disease or cerebral vascular disease (Kim *et al.* 2008). Two cross-sectional studies with mean blood Pb levels >10 µg/dL do support a relationship between blood Pb and coronary heart disease and stroke (Pocock *et al.* 1988, Schwartz 1991). In cross-sectional studies of peripheral artery disease, risk increased with blood Pb in NHANES 1999-2002 studies with very low Pb levels (mean, 1.6-2.1 µg/dL) (Navas-Acien *et al.* 2004, Muntner *et al.* 2005, Guallar *et al.* 2006), independent of an unadjusted association with homocysteine level and accounting for renal function (Guallar *et al.* 2006).

Several studies used vascular measures as indicators of arterial function in the absence of physician-diagnosed disease, but there was inadequate information to form a conclusion because each was only studied for an association with Pb exposure in one group. In a study of Japanese ceramics workers, blood Pb (mean, 13 µg/dL) was associated with decreased finger blood flow in response to a postural change, consistent with an atherosclerotic effect, but four other measures of cardiac function were not associated (Ishida *et al.* 1996). In a study of bus drivers with low blood Pb levels (mean, 6 µg/dL), mean aging index of second derivative finger photoplethysmogram waveform (SDPTG-AI) was increased with blood Pb, indicating lower central and peripheral arterial function (Kaewboonchoo *et al.* 2010). In otherwise healthy young women with very low blood levels (mean not reported, but almost all subjects <1 µg/dL), intima-media thickness of the common and carotid arteries was increased with blood Pb, while there was no association with increases in eight other metals tested (Zeller *et al.* 2010). The associations of blood Pb with these tests of cardiovascular functions have not been replicated, but they suggest a role for Pb in early hallmarks of impaired cardiovascular function without a diagnosis of clinical cardiovascular disease.

Summary of support for conclusions on clinical cardiovascular disease

The EPA's 2006 AQCD presents a small animal literature supporting an atherogenic effect of chronic Pb exposure, as well as impacts on vascular tissue and smooth muscle cells (see U.S. EPA 2006 for further review of these studies). They reported effects of Pb as conducive to thrombosis, hyperlipidemia, arteriosclerosis, and vascular remodeling. The human data are consistent with these findings but diverse in scope, hampering the ability to form conclusions on specific cardiovascular disease endpoints.

The NTP's conclusion of *limited* evidence for clinical cardiovascular disease is based on a heterogeneous group of related cardiovascular outcomes in studies that mostly found significant effects associated with blood Pb <5 µg/dL.

6.3.5 Cardiovascular Mortality

There is *limited* evidence that blood Pb levels <10 µg/dL are associated with increased mortality from cardiovascular causes (see **Table 6.7** and Cardiovascular Mortality section of Appendix C: Cardiovascular Effects). A association between increased cardiovascular mortality and increased blood Pb was supported by three prospective studies but was not supported by two prospective studies, one of which reported a significant association with bone Pb. One of the supportive studies had a mean blood Pb level above 10 µg/dL (12.64 µg/dL) (Moller and Kristensen 1992), and one reported mean blood Pb levels of 2.58 µg/dL (Menke *et al.* 2006).

Large studies that found associations between Pb and BP also indicated an increased risk of mortality from cardiovascular causes. In NHANES III, after 12 years of follow-up using the National Death Index, there was increased mortality associated with baseline blood Pb levels (Menke *et al.* 2006, Schober *et al.* 2006). In the Normative Aging Study, bone Pb, but not blood Pb at mean Pb level of 5.6 µg/dL, was associated with BP and cardiovascular mortality (Weisskopf *et al.* 2009). In the Glostrup Population Study, there was no association between blood Pb and BP, cardiovascular disease, or total mortality after 14 years of follow-up (Moller and Kristensen 1992). While Pb levels were not associated with incidence of myocardial infarction, subjects who died of a third myocardial infarction had significantly higher hair Pb levels (Afridi *et al.* 2010).

Summary of support for conclusions on cardiovascular mortality

Mortality from cardiovascular causes is not addressed in the EPA's 2006 AQCD summary of the animal data. The conclusion of *limited* evidence for Pb in cardiovascular mortality is based on consideration of the three studies (two of which used the same NHANES III sample) with a significant effect of blood Pb and the two studies that did not find an association with blood Pb levels. The NTP's conclusions for *limited* evidence for cardiovascular mortality at blood Pb levels <10 µg/dL expands upon the conclusion from ATSDR's Toxicological Profile (ATSDR 2007) on increased cerebrovascular mortality.

Table 6.7: Studies of Pb and cardiovascular mortality used to develop conclusions				
Relevance to Conclusions	**Study Description**	**Study Design**	**Key Cardiovascular Findings**	**Reference**
NHANES III				
Effect	13,946 adults	Prospective	Mortality was increased with higher Pb levels for death from all causes, cardiovascular disease, myocardial infarction, and stroke—but not cancer—for these adults from the general population with low blood Pb levels (geometric mean=2.58 µg/dL) and up to 12 years of follow-up.	Menke (2006)
	9,757 adults >40 years old	Prospective	In this cohort of older NHANES III participants followed for up to 12 years, blood Pb was associated with higher mortality from all causes, cardiovascular disease, and cancer, at blood Pb levels of 5-10 µg/dL.	Schober (2006)
No effect	1,052 adults, 14-year follow-up, Glostrup Population Study, Denmark	Prospective	In adults with 11.5 µg/dL mean blood Pb at 40 years old, there was no association with all-cause mortality after 14 years of follow-up, while there were reductions in blood Pb levels over this time.	Møller (1992)
Effect	927 dialysis patients, Taiwan, 18 months of follow-up	Prospective	In these hemodialysis patients, baseline blood Pb >12.64 µg/dL (median, 16.4 µg/dL) was associated with higher all-cause, cardiovascular cause, and infection cause mortality over 18 months of follow-up.	Lin (2011)
Effect	860 men in NAS with bone Pb and 1,235 with blood Pb (1994) and follow-up (2007)	Prospective	Bone Pb >35 µg/g increased the risk of mortality from all causes and from cardiovascular causes, but not cancer mortality. Blood Pb was not associated with mortality (highest >6 µg/dL).	Weisskopf (2009)

Epidemiological studies of Pb exposure and cardiovascular mortality are listed by decreasing size, grouped together for overlapping or shared study groups. Abbreviations: NAS, Normative Aging Study (Boston area, began in 1963 by the Veterans Administration); NHANES, National Health and Nutrition Examination Survey.

6.4 Susceptible Populations and Modifiers of Pb Exposure

Segments of the population that are more susceptible to health effects of Pb are discussed more extensively in **Section 3.0 Exposure**. It is unknown whether chronic exposure and other cardiovascular risk factors can modify the relationship between Pb and BP, putting some portions of the population at greater risk of cardiovascular disease. These other factors may also impair the ability to detect an association of Pb with BP in some general population studies, even those with higher Pb levels. This concept was proposed by Orssaud *et al.* (1985): "The increase in blood lead concentration parallels the increase in blood pressure until some limit value, so that such a trend is apparent only when other factors (such as age) do not competitively increase blood pressure by greater amounts."

Susceptible Populations: Pb exposure may disproportionately affect populations with preexisting conditions that can be exacerbated by a small increase in BP (see **Section 7.0 Renal Effects**). A prospective study of dialysis patients found significantly increased cardiovascular mortality over 18 months of follow-up for the middle range of Pb levels (second tertile, 8.5-12.6 µg/dL: HR=3.7 (95% CI: 2.1, 6.5)) and high levels of Pb (third tertile, >12.6 µg/dL: HR=9.7 (95% CI: 2.1, 23.3)) compared to the low Pb range (first tertile, <8.5 µg/dL) (Lin *et al.* 2011).

As discussed in **Section 6.3.1 Blood Pressure (BP) and Hypertension**, pregnant women, menopausal women, and children may be at greater risk of Pb related increases in BP (see further discussion in **Section 3.4 Modifiers of Pb Exposure**). The evidence for an effect of Pb on BP in children is limited, but blood Pb at 2 years of age was also associated with ECG abnormalities at 9.5 years of age, particularly in subjects with lower socioeconomic status (Gump *et al.* 2005, Gump *et al.* 2007).

Modifiers: Age is associated with higher BP and increased risk of cardiovascular disease, and Pb levels are also strongly correlated with age (Den Hond *et al.* 2002). It is unclear if this is an effect of higher Pb exposures in earlier eras in this group, or if the elderly are more susceptible to cardiovascular effects of Pb (see further discussion in **Section 3.4 Modifiers of Pb Exposure**). Many studies adjusted for age in their analyses, while others did analyses within specific age groups (stratified analyses). One study of subjects over age 75 years of age found no association between blood Pb and BP (Nordberg *et al.* 2000).

Gender and ethnicity are also correlated with Pb exposure and cardiovascular risk and are frequently adjusted for in analyses. Many general population studies found higher blood Pb, higher BP, and more hypertension in non-Caucasian groups (Rothenberg *et al.* 1999, Den Hond *et al.* 2002, Scinicariello *et al.* 2010). Men generally have higher blood Pb and BP than do women (Staessen *et al.* 1990, Hense *et al.* 1993, 1994, Chu *et al.* 1999, Den Hond *et al.* 2002). Many occupational studies only included male subjects, even in those with low occupational Pb exposure (mean Pb levels, <10 µg/dL) (Orssaud *et al.* 1985, Sharp *et al.* 1990, Schuhmacher *et al.* 1994, Wolf *et al.* 1995, Sirivarasai *et al.* 2004, Kaewboonchoo *et al.* 2007), while only the Nurses' Health Study focused on female workers (Korrick *et al.* 1999). Numerous other studies included gender in their adjustment factors.

Genetic variation is an important biological factor that can characterize a susceptible population and modify the relationship between an exposure and disease via altered absorption or metabolism. Unlike other relevant cofactors such as gender and age group, genotype is almost always blinded from subjects and investigators and so is less likely to bias exposure or ascertainment. In the three studies that included genetic variants in evaluation of Pb and cardiovascular effects, the genotyped polymorphisms were not independent risk factors for increased pulse pressure, hypertension, or QT prolongation, but carriers of genetic variants had stronger associations between Pb and effects (Park *et al.* 2009a, Scinicariello *et al.* 2010, Zhang *et al.* 2010). These genetic modifications of the effect of Pb on cardiovascular outcomes have not been sufficiently studied to judge their reproducibility. However, if genetic variants can modify the impact of Pb exposure, it supports a biological basis for associations between Pb and effects beyond what might spuriously arise by unmeasured confounding factors.

Dietary factors may also modify the relationship between Pb and BP. People who drank alcohol had higher blood Pb and stronger associations between Pb and BP (Hense *et al.* 1994, Menditto *et al.* 1994, Pizent *et al.* 2001). Among black male bus drivers, infrequent caffeine users had a stronger positive relationship between blood Pb and BP than did habitual users (Sharp *et al.* 1990). There is some indication from the literature that there is an interaction between Pb and calcium intake (Dolenc *et al.* 1993), with low calcium related with higher blood Pb (Pizent *et al.* 2001) and a higher risk of hypertension (Elmarsafawy *et al.* 2006). However, a 12-week calcium supplement intervention had no effect on blood Pb, indicating that any effect of calcium on BP is not via interaction with Pb (Morris *et al.* 1990).

6.5 Conclusions

The NTP concludes that there is *sufficient* evidence for a small, but detectable, increase in BP associated with Pb exposure in groups with mean blood Pb levels <10 µg/dL (see **Table 6.8** for complete list of cardiovascular effects conclusions). There is *sufficient* evidence for an increase in BP and hypertension during pregnancy at levels <10 µg/dL. There is *limited* evidence for increased mortality from cardiovascular causes from Pb levels <10 µg/dL. There is *limited* evidence of an increase in early markers of impaired cardiac function (ECG abnormalities) and cardiovascular disease in general. There is *inadequate* evidence to evaluate Pb effects at levels <10 µg/dL on heart rate variability, specific cardiovascular morbidities, or any cardiovascular effects in children. There is *inadequate* evidence to evaluate Pb effects at levels <10 µg/dL on hypertension or other cardiovascular diseases in menopausal women.

Bone Pb reflects chronic Pb exposure and is more consistently associated with increased BP, hypertension, cardiovascular disease, and mortality. Not all human studies conducted at low levels of exposure support these associations. The animal data strongly support a causative relationship, even at low levels relevant to human exposure (e.g., BP increases in rats with blood Pb as low as 2.15µg/dL Tsao *et al.* 2000). The observed heterogeneity across the human literature may be partially explained by variation in biologically susceptible groups with other Pb-related risk factors for cardiovascular disease, such as age, alcohol, caffeine, or calcium intake, or genetic polymorphisms in metabolic genes. Menopausal women and children may

also be at increased risk of cardiovascular effects from Pb exposure, but they have been inadequately studied.

Table 6.8: Conclusions on cardiovascular effects of low-level Pb

Health Effect	Population	Conclusion	Blood Pb Evidence	Bone Pb Evidence
Blood pressure and hypertension	Adults	*Sufficient*	Yes, <10 µg/dL	Yes
	Children	*Inadequate*	Unclear	Yes (one study)
	Pregnant women	*Sufficient*	Yes, <10 µg/dL	Not studied
	Menopausal women	*Inadequate*	Unclear	Not studied
Heart rate variability	Adults	*Inadequate*	Unclear	Yes (one study)
Electrocardiogram abnormalities	Men	*Limited*	No	Yes (one study)
	Children	*Limited*	Yes, <5 µg/dL (one study)	Not studied
Clinical cardiovascular disease (general)	Adults	*Limited*	Yes, <5 µg/dL	Yes (one study)
Clinical cardiovascular disease (specific)	Adults	*Inadequate*	Unclear	Yes (one study)
Cardiovascular mortality	Adults	*Limited*	Yes, <10 µg/dL	Yes (one study)

7.0 RENAL EFFECTS

7.1 Conclusions

The NTP concludes that there is *sufficient* evidence that blood Pb levels <5 µg/dL in adults are associated with adverse effects on kidney function adults. With few exceptions, epidemiological studies of the general population reported associations between blood Pb levels <10 µg/dL and (1) increased risk of chronic kidney disease (CKD) and (2) decreased estimated glomerular filtration rate (eGFR) and creatinine clearance. The associations are typically stronger in people with hypertension or diabetes (Muntner *et al.* 2003, Tsaih *et al.* 2004). The NTP recognizes that an individual with blood levels <10 µg/dL during adulthood may have had higher blood Pb levels earlier in life, and the role of early-life Pb exposure cannot be discriminated from the role of concurrent blood Pb. Comparatively few studies examined markers of exposure other than blood Pb levels; therefore, it is unknown if blood or bone Pb levels are more consistently associated with kidney effects.

The data are *inadequate* to evaluate whether prenatal exposure to Pb is associated with impaired kidney function later in life. No studies were identified that evaluated prenatal blood Pb and kidney function in children or adults. Relatively few studies have assessed kidney measures in children in association with low-level Pb exposure. The findings from these studies are less consistent than are studies in adults. Most of these studies also use kidney biomarkers, whose prognostic value for renal function is less clear compared to GFR measures and biomarkers, such as microalbuminuria, commonly measured in the adult studies. Thus, there is currently *inadequate* evidence to conclude that blood Pb <10 µg/dL is associated with impaired kidney function in children <12 years old. In contrast, there is *limited* evidence that blood Pb levels <5 µg/dL are associated with adverse effects on kidney function in children ≥12 years old. A recent study of children and young adults 12-20 years of age in NHANES 1988-1994 with mean blood Pb of 1.5 µg/dL reported a reduction in eGFR rate per doubling of blood Pb (Fadrowski *et al.* 2010). The reduction in GFR demonstrated in Fadrowski *et al.* (2010) is consistent with results from adults within NHANES and supports adverse effects on kidney function in children age 12 and over at blood Pb <5 µg/dL.

Although the cardiovascular and renal systems are intimately linked, effects are considered separately in this evaluation because studies generally reported individual effects rather than testing both systems comprehensively. Nevertheless, it is recognized that hypertension can contribute to adverse renal effects and that kidney dysfunction can contribute to increased blood pressure (BP) and hypertension. Cardiovascular and renal effects of Pb may share the same biological mechanisms (U.S. EPA 2006, ATSDR 2007). As discussed in **Section 7.4.2,** the human literature supports a strong association between BP and renal effects of Pb, and multiple studies suggest that people with hypertension are a susceptible population for adverse renal effects of Pb. This interrelationship of effects is also supported in animal models; for example, Pb exposure accelerates chronic renal disease by raising BP in male rats (Roncal *et al.* 2007).

7.2 How Conclusions Were Reached

Conclusions in the NTP evaluation of Pb-related kidney effects in humans associated with low-level Pb are derived from epidemiological studies with a focus on blood Pb levels <10 µg/dL. For this evaluation we did not consider studies with mean blood Pb >15 µg/dL, because in those studies, the subjects with Pb <10 µg/dL are often used as the reference group and are not appropriate for evaluating low-level Pb effects. This evaluation focuses on the human data for kidney effects of Pb because there is a relatively large database of human studies for these effects; therefore, the document makes only limited use of the data from laboratory animals to support the human evidence. Major endpoints considered as potential indicators of kidney effects of Pb are listed and briefly described in **Section 7.2.1**. This document is not a review of kidney toxicity, and the reader is directed to published reviews for additional background. Key data and principal studies considered in developing the NTP's conclusions are discussed in detail in **Section 7.3 Evidence for Pb-related Effects on Kidney Function**. The discussion of each kidney effect begins with a statement of the NTP conclusion whether the specific effect is associated with a blood Pb level <10 µg/dL or <5 µg/dL and the age group in which it is identified (childhood, or adulthood), as well as the timing of exposure associated with the effect (prenatal, childhood, concurrent) when available. Although the information necessary to support the NTP's conclusions is presented in **Section 7.3**, the complete data set of human studies considered for evaluation of kidney effects of low-level Pb is included in Appendix D: Renal Effects, and individual studies are abstracted for further reference. The NTP made extensive use of recent government evaluations of health effects of Pb in the current assessment, and the relevant conclusions of the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) are briefly described in **Section 7.2.2** below.

7.2.1 Principal Measures of Kidney Effects

Table 7.1 lists a number of kidney endpoints commonly evaluated in epidemiological studies. The most clinically accepted measure of kidney function is glomerular filtration rate (GFR), which is the flow rate of filtered fluid through the kidney. The gold standard method of determining GFR is through the use of radionuclide or radiocontrast markers, which is both costly and time consuming. GFR can be approximated by creatinine clearance, which compares creatinine levels in blood and urine to calculate the volume of blood plasma cleared per milliliter of creatinine per unit time. Direct measurement of creatinine clearance requires 24-hour urine collection. There are also a number of equations for estimating GFR or creatinine clearance based on serum biomarkers (e.g., creatinine, cystatin C) and consideration of other variables such as age, sex, race, or weight. Historically, serum creatinine has been used most often, although serum cystatin C is increasingly being used as an alternative or complementary approach to serum creatinine for estimating GFR.

GFR is notoriously insensitive (Levey *et al.* 1999), and "early biological effect markers" (EBEs), such as N-acetyl-β-D-glucosaminidase (NAG), are thought to be more sensitive because they are

Table 7.1: Commonly used indicators of kidney function in the Pb literature references

Kidney Endpoint	Measurement	Description	Indication of Impaired Kidney Function
Clinical Indicators of impaired kidney function			
Glomerular filtration rate (GFR)	Serum creatinine (most common)	Breakdown product of creatine phosphate in muscles; commonly used measure of GFR (considered less precise than cystatin C); can be influenced by non-kidney function variables that affect muscle mass (gender, age, race, weight, diet)	↑serum concentration
	Serum cystatin C	A cysteine protease inhibitor protein used as an alternative to serum creatinine or complementary measure to estimate GFR	↑ serum concentration
	eGFR (estimated GFR, based on equations)	Modification of Diet in Kidney Disease (MDRD) Study: Clinical standard of estimated GFR based on creatinine but underestimates at levels in normal range CKD-Epidemiology Collaboration (CKD-EPI): More recent way to estimate GFR based on creatinine; better accuracy in normal range than MDRD	↓ eGFR
	Creatinine clearance (based on timed urine collections)	Cockcroft-Gault: Oldest, estimates creatinine clearance	↓ creatinine clearance
	Blood urea nitrogen	Measures the amount of nitrogen in blood in the form of urea (a waste product of protein digestion)	↑serum concentration
	^{125}I-iothalamate, iohexol, and other radioisotopes	Radioactive markers used to measure GFR by timed sequential blood samples or imaging (invasive and time consuming)	↓ urinary clearance
Indicators of early biological effect markers (EBEs)			
Function	β_2-microglobulin (only validated EBE marker)		↑ urine concentration
	Total protein, albumin, and low- to intermediate-molecular-weight proteins (e.g., retinol-binding protein (RBP)), Clara cell protein, transferring)		↑ urine concentration
Biochemical or histological alteration	Biochemical: urinary eicosanoids (e.g., prostaglandin E_2 (PGE2), prostaglandin $F_{2\ alpha}$, 6-keto-prostaglandin $F_{1\ alpha}$ (6-keto-PGF), and thromboxane B_2 (TXB2)), fibronectin		Varies/not necessarily known[1] (e.g., ↓ urine PGE2, 6-keto-PGF; ↑ TXB2)
	Histological: brush border antigen, fibronectin (glomerular fibrosis)		
Cytotoxicity	N-acetyl-β-D-glucosaminidase (NAG)	Lysosomal enzyme involved in the breakdown of glycoproteins	↑ activity
Other			
	Urate/uric acid	Urate is a salt derived from uric acid; can build up in the body when uric acid is not adequately metabolized, e.g., in cases of gout	↑ urine concentration

[1](Cardenas *et al.* 1993, Rose and Post 2011).

often elevated when GFR measures are not abnormal. However, the validity and reliability of EBEs for long-term prognostic value are unclear. The epidemiological studies in children more often assess EBEs for kidney rather than eGFR or creatinine clearance. It should also be noted that the list of biomarkers of EBEs presented in **Table 7.1** is not comprehensive and reflects those most commonly reported in the Pb epidemiological studies. Other biomarkers of kidney function continue to be assessed within kidney epidemiology research, especially for acute kidney injury, with recent attention focusing on urinary proteins such as neutrophil gelatinase-associated lipocalin, kidney injury molecule-1, and liver-type fatty acid binding protein (Devarajan 2010, Tesch 2010).

7.2.2 Principal Conclusions from the 2006 EPA and 2007 ATSDR Pb Documents

The EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) both concluded that epidemiologic studies support a relationship between Pb exposure kidney effects at lower blood Pb levels (**Table 7.2**). The EPA states that most studies in general adult and patient populations published between 1986 and 2006 indicate that Pb, at much lower doses than those causing Pb nephropathy, acts as a cofactor with other more established renal risks to increase the risk for renal dysfunction. Other explanations, such as residual confounding or reverse causality, are not likely to account for the observed associations between Pb dose and kidney dysfunction. It should be noted that the EPA is in the process of revising the AQCD, and the conclusions of the external draft (U.S. EPA 2012) are largely in line with the 2006 AQCD plus additional review of the evidence for reduced kidney function at low blood Pb.

Table 7.2: Main conclusions for kidney effects in 2006 EPA AQCD for Lead and the 2007 ATSDR Toxicological Profile for Lead

"General population studies are the most important advance in this regard. These studies provide strong evidence that renal effects occur at much lower blood Pb levels than previously recognized. These effects are clinically relevant in U.S. subpopulations who continue to have higher Pb exposure than the general population. At levels of exposure in the general U.S. population overall, Pb combined with other risk factors, such as diabetes, hypertension, or chronic renal insufficiency from non-Pb related causes, can result in clinically relevant effects. Notably, the size of such susceptible populations is increasing in the US due to obesity… The threshold for Pb-related nephrotoxicity cannot be determined based on current data. However, associations with clinically relevant renal outcomes have been observed in populations with mean blood Pb levels as low as 2.2 µg/dL." (U.S. EPA 2006, pg 6-113)
"The overall dose-effect pattern suggests an increasing severity of nephrotoxicity associated with increasing PbB [blood Pb level], with effects on glomerular filtration evident at PbBs below 20 µg/dL, enzymuria and proteinuria becoming evident above 30 µg/dL, and severe deficits in function and pathological changes occurring in association with PbBs exceeding 50 µg/dL." (ATSDR 2007, pg 79)

The NTP considered the conclusions and data summaries from the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007). In general, the NTP concurred with the conclusions and agreed that the data support them.

7.3 Evidence for Pb-related Effects on Kidney Function

7.3.1 Kidney Effects in Adults

There is *sufficient* evidence available for an association between current blood Pb levels <5 µg/dL in adults, measured at the time of the study, and reduced kidney function in general populations (**Table 7.3**, see also Appendix A: Neurological Effects). Associations between low-level blood Pb and impaired kidney function have been reported in studies assessing participants in NHANES (Muntner *et al.* 2003, Muntner *et al.* 2005, Navas-Acien *et al.* 2009), the Normative Aging Study (Payton *et al.* 1994, Kim *et al.* 1996, Tsaih *et al.* 2004), the Swedish Women's Health in the Lund Area (WHILA) study (Akesson *et al.* 2005), and native and nonnative residents in rural Taiwan (Lai *et al.* 2008). The blood Pb levels associated with kidney effects in these studies were ≤10 µg/dL and <5 µg/dL in the NHANES and WHILA studies.

There is no apparent threshold for kidney effects. Significant increases in risk of CKD based on an eGFR <60 mL/min/1.73 m^2 have been reported in NHANES for blood Pb levels of >1.63 µg/dL (adjusted OR=1.89 (95% CI: 1.09, 3.30) (Muntner *et al.* 2005)) and >2.4 µg/dL (adjusted OR=1.56 (95% CI: 1.17, 2.08) (Navas-Acien *et al.* 2009)). Similarly, blood Pb levels of ≤5 µg/dL in the WHILA study (median, 2.2 µg/dL; 5th to 95th percentile, 1.1-4.6 µg/dL) were significantly associated with reduced eGFR (adjusted β=-0.2 (95% CI: -0.32, -0.09) (Akesson *et al.* 2005)). No significant associations with blood Pb and either serum creatinine or creatinine clearance were observed in a study of 709 men in the Normative Aging Study (β coefficients not reported) (Wu *et al.* 2003); however, this study did report a significant association between higher patella Pb and lower creatinine clearance. Higher blood Pb levels were associated with lower creatinine clearance in WHILA participants (adjusted β=-0.18 (95% CI: -0.3, -0.06)). The EPA's 2006 AQCD for Lead (U.S. EPA 2006) considered the clinical significance of these findings. An increase in blood Pb reported in Akesson *et al.* (2005) of 3.5 µg/dL from the 5th percentile (1.1 µg/dL) to the 95th percentile (4.6 µg/dL) had the same effect on glomerular filtration as an increase in age of 4.7 years or 7 kg/m^2 in body mass index (BMI) (U.S. EPA 2006). A 10-fold increase from 1 to 10 µg/dL would result in a 16.2 mL/min decrease in estimated creatinine clearance (U.S. EPA 2006). As discussed below under **Section 7.4 Susceptible Populations or Life stages**, the impacts of Pb on kidney function in people with diabetes, hypertension, or CKD from non-Pb related causes are expected to be higher (U.S. EPA 2006).

Many of the studies that support an association between blood Pb and kidney outcomes included statistical adjustments for factors such as age and sex, with studies based on the Normative Aging Study, NHANES, WHILA, and rural Taiwanese groups including additional variables for smoking status and/or alcohol consumption. The significant associations remaining after adjustment suggest that these factors were not sufficient to account for the

Table 7.3: Studies of kidney outcomes in adults				
Relevance to Conclusions	**Study Description**	**Study Design**	**Key Kidney Findings**	**Reference**
Normative Aging Study, USA				
Effect	Men 43-90 years old (n=744)	Cross-sectional	Creatinine clearance (natural log (Ln)) was inversely associated with Ln blood Pb (adjusted β (SE) =-0.0403 (0.0198) µg/dL; p=0.0426). Average blood Pb, 8.1 µg/dL (range, <5 to 26 µg/dL).	Payton (1994)
Effect	Men 34-88 years old (n=459)	Prospective	Blood Pb ≤10 µg/dL positively associated with concurrent serum creatinine (β (SE)= 0.060 (0.019); p=0.002), but not with a change in serum creatinine (β (SE)= 0.039 (0.025); p=0.13).	Kim (1996)
Effect	Men average age of 66 years followed for 6 years (n=448)	Prospective	Significant association with change in serum creatinine with baseline blood Pb in diabetics (adjusted β (SE)=0.076 (0.023); p<0.05) but not nondiabetics (adjusted β (SE)=0.006 (0.005); p=not significant). Average baseline blood Pb, 6.5 µg/dL.	Tsaih (2004)
Cadmibel, Belgium				
Effect	Adults 20-88 years old participating in the Cadmibel study (n=1,981)	Cross-sectional	10-fold increase in blood Pb associated with reduction in creatinine clearance of 10 mL/min (female) to 13 mL/min (male); adjusted OR (95% CI) for 10-fold increase in blood Pb and impaired kidney function = 3.76 (1.37, 10.4). Average (range) blood Pb of 11.4 (2.3, 72.5) µg/dL in males and 7.5 (1.7, 60.3) µg/dL in females.	Staessen (1992)
NHANES, USA				
Effect	Adults >20 years old included in NHANES 1988-1994 (n=15,211 total; 4,813 hypertensives)	Cross-sectional	Increased risk in hypertensives (but not normotensives) for elevated serum creatinine (Q2 (2.5-3.8 µg/dL) vs. Q1 (0.7-2.4); adjusted OR (95% CI) =1.47 (1.03, 2.10)) and CKD (Q3 (3.9-5.9 µg/dL) vs. Q1 (0.7-2.4); adjusted OR (95% CI)=1.85 (1.32, 2.59)).	Munter (2003)
Effect	Adults ≥20 years of age included in NHANES 1999-2006 (n=14,778)	Cross-sectional	Risk of having a reduced GFR (defined at <60 mL/minute/1.73 m^2) was higher for blood Pb of >2.4 µg/dL vs. ≤1.1 µg/dL (adjusted OR=1.56 (95% CI: 1.17, 2.08); p_{trend}<0.001); significant trend for albuminuria (p_{trend}≤0.001).	Navas-Acien (2009)
Effect	Adults 18-75 years old from NHANES 1999-2002 (n=9,961)	Cross-sectional	Increased risk for CKD (GFR <60 mL/min) associated with blood Pb of >1.63 µg/dL (Q3 (1.63-2.47 µg/dL) vs. Q1 (<1.06 µ µg/dL); adjusted OR (95% CI)=1.89 (1.09, 3.30)).	Muntner (2005)
Patients				
Effect	CKD Taiwanese patients 25-82 years old followed for 4 years (n=121)	Prospective	1 µg/dL higher blood Pb at baseline associated with a 4.0 mL/min/1.73 m^2 reduction in eGFR over 4 years. Average (range) blood Pb, 4.2 µg/dL (1-13.4 µg/dL).	Yu (2004)
Effect	Chronic renal insufficiency patients 25-80 years old followed for 2 years (n=202)	Prospective	Baseline chelatable Pb was significantly associated with risk for achieving an increase in serum creatinine to 1.5 times baseline (HR (95% CI)=1.00 (1.00, 1.01)). Average (range) blood Pb at baseline, 5.3 (0.6-16.1) µg/dL; 31 patients on chelation therapy during months 24-51 had better GFR outcomes.	Lin (2003)

Table 7.3: Studies of kidney outcomes in adults				
Relevance to Conclusions	**Study Description**	**Study Design**	**Key Kidney Findings**	**Reference**
Effect	CKD patients 30-80 years old followed for 2 years	Prospective	Baseline chelatable Pb was significantly associated with risk for achieving an increase in serum creatinine to 1.5 times baseline (HR (95% CI)=1.03 (1.00, 1.07)). Average (range) blood Pb at baseline, 2.9 (0.8-10.3) µg/dL; 16 patients on chelation therapy during months 24-51 had better GFR outcomes.	Lin (2006a)
Effect	Diabetes patients 33-79 years old followed for 1 year	Prospective	Baseline chelatable Pb was significantly associated with risk for achieving an increase in serum creatinine to 1.5 times baseline (HR (95% CI)=1.01 (1.01, 1.02)). Average (range) blood Pb at baseline, 6.5 (1.9-19.1) µg/dL; 15 patients on 3-month chelation therapy during months 13-24 had better GFR outcomes.	Lin (2006b)
Other				
Effect	Adult women from WHILA study in Sweden; n=820	Cross-sectional	Inverse association with GFR (β (95% CI)=-0.20 (-0.32, -0.09)) and creatinine clearance (β (95% CI)=-0.18 (-0.30, -0.06)). Mean (5-95% percentiles) blood Pb, 2.2 (1.1, 4.6) µg/dL. No association with NAG or α1-microglobulin.	Akesson (2005)
Effect	Adult native and nonnative rural Taiwanese (n=2565)	Cross-sectional	Increased risk of serum creatinine >1.2 mg/dL for blood Pb >7.5 versus ≤ 7.5 µg/dL (adjusted OR=1.92 (95% CI: 1.18, 3.10)).	Lai (2008)
No effect	Adults 18-51 years old living near two smelters in France; n=300 and 300 age/gender-matched referents	Cross-sectional	No difference in any kidney parameters in adults living in reference area (average blood Pb: males, 7.1 µg/dL; females, 4.2 µg/dL) and polluted area (average blood Pb: males, 6.8 µg/dL; females: 5.3 µg/dL) (serum creatinine, total protein, albumin, transferrin, β_2-microblobulin, RBP, brush border antigen, and NAG).	de Burbure (2003)
Equivocal	Men 40-59 years old participating in the Regional Heart Study in England (n=7,364)	Cross-sectional	Blood Pb associated with log-transformed serum urate (β=0.06) and serum urea (β=-0.05); no association with serum creatinine. Blood Pb levels ranged from <12.4 to 37.3 µg/dL (authors considered the magnitude of the changes to be small and unlikely to be of biological importance)	Pocock (1984)
Equivocal	Adult London civil servants in England 37-58 years old (n=531)	Cross-sectional	In males, significant correlation between serum creatinine and log blood Pb (r=0.10, p=0.04). In females, no correlation with serum creatinine and log blood Pb (r=0.03, p=not significant).	Staessen (1990)
No effect	Men 25-38 years old in Egypt (n=35 smokers, 33 nonsmokers)	Cross-sectional	No significant correlations were found between Pb and markers of kidney damage in smokers (14.4 µg/dL) or nonsmokers (10.2 µg/dL).	Mortada (2004)

Epidemiological studies of blood Pb exposure and kidney function are listed by study are listed by study type and decreasing study size, grouped together for overlapping or shared study groups.

Abbreviations: CI, confidence interval; CKD, chronic kidney disease; GFR, glomerular filtration rate; HR, hazard ratio; NAG, N-acetyl-β-D-glucosaminidase; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; Q1, first quartile; Q2, second quartile; Q3, third quartile; RBP, retinol-binding protein; SE, standard error; WHILA, Women's Health in the Lund Area.

observed associations between blood Pb and kidney outcomes (Payton *et al.* 1994, Kim *et al.* 1996, Muntner *et al.* 2003, Tsaih *et al.* 2004, Akesson *et al.* 2005, Muntner *et al.* 2005, Lai *et al.* 2008, Navas-Acien *et al.* 2009). Most of these studies also included blood pressure (BP) or hypertension status as an adjustment factor, which is expected to underestimate the association between Pb exposure and kidney effects (i.e., bias towards the null) given the positive relationship that exists between Pb and BP in the general population (U.S. EPA 2006). The studies that were considered equivocal or not supportive of an association typically assessed kidney effect by measurement of serum creatinine and did not consider the impact of potential or modifying variables at all (de Burbure *et al.* 2003) or to the same extent as the studies cited above that assessed GFR or creatinine clearance (Pocock *et al.* 1984, Staessen *et al.* 1990, Mortada *et al.* 2004). An additional limitation to interpretation of the nonsupportive findings of de Burbure *et al.* (2003) is that the average blood Pb levels between the "exposed" and reference groups were quite similar and actually higher in men in the reference group than in men considered exposed based on living near a smelter for ≥8 years (average blood Pb in referents: males, 7.1 µg/dL; females, 4.2 µg/dL; vs. "exposed": males, 6.8 µg/dL; females, 5.3 µg/dL).

Most of the studies summarized in **Table 7.3**, were cited in the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and considered in relation to potential reverse causality, which would occur if impaired kidney excretion leads to less efficient elimination of Pb, and thus higher estimates of internal Pb exposure, resulting in a bias towards detecting associations between Pb and impaired kidney function. The EPA did not consider potential reverse causality to be sufficient to explain the associations between Pb and kidney outcomes (U.S. EPA 2006). There are three lines of evidence that argue against reverse causality as an explanation linking higher blood Pb levels and reduced kidney function: (1) there is no experimental evidence supporting reverse causality; (2) there is evidence against reverse causality; and (3) there is no evidence that renal failure changes blood Pb concentrations. Evidence against reverse causality is found in (1) direct bone biopsies, (2) EDTA (ethylenediaminetetraacetic acid) chelation tests, and (3) blood Pb measurement performed in subjects without renal failure, with renal failure of known non-Pb etiology, and with chronic Pb nephropathy, indicating that renal failure per se does not increase bone Pb stores or blood Pb and does not mobilizable Pb (Van de Vyver *et al.* 1988). The absence of excessive Pb retention caused by renal failure is further supported by the finding that EDTA mobilizable Pb in patients with Pb-associated renal failure is the same as in subjects without renal failure (Emmerson 1973, Batuman *et al.* 1983). Additional data against reverse causality comes from the longitudinal study by Yu *et al.* (2004) of CKD patients in Taiwan, where both baseline blood and EDTA-chelatable Pb levels predicted kidney function decline over 4 years. Also, two publications from the Normative Aging Study report associations between blood Pb and serum creatinine across the entire range of serum creatinine (Kim *et al.* 1996, Tsaih *et al.* 2004), including at levels in the normal range where reverse causality would not be occurring. Other evidence considered in the EPA's 2006 AQCD for Lead (U.S. EPA 2006), cited as an April 12, 2006, personal communication from Agneta Åkesson to Virginia Weaver, is that higher urine Pb was associated with lower estimated creatinine clearance in Swedish women in the WHILA study. Urinary excretion of Pb should decrease as kidney function declines.

Summary of support for conclusions

Data from animal studies provide strong support for an association of Pb-associated kidney toxicity, including histopathological changes and decreased glomerular filtration rate (GFR), with chronic exposure that results in high blood Pb levels (>40µg/dL in rats; seeU.S. EPA 2006 for recent reviews of the animal data, ATSDR 2007). Rodent studies also support hyperfiltration, or increased GFR as part of an early phase of kidney injury (e.g., Khalil-Manesh *et al.* 1992b). Many in vivo and in vitro studies support oxidative stress as the mechanism by which Pb contributes to the pathogenesis of kidney disease (see U.S. EPA 2006 for further review of animal and mechanistic studies). Although animal data support a role for oxidative stress and a potential role for metallothionein in kidney effects associated with Pb exposure, the animal studies are generally at higher Pb exposure levels (20-60 µg/dL) than the blood Pb levels associated with decreased GFR in humans (<10 µg/dL). The human data include multiple studies that reported an association between blood Pb levels <10 µg/dL and increased risk of CKD, as well as inverse associations with GFR and creatinine clearance in the general population and even stronger evidence in people with hypertension or diabetes. A number of studies reported effects at blood Pb levels in the 2-3 µg/dL range (e.g., Akesson *et al.* 2005, Muntner *et al.* 2005, Navas-Acien *et al.* 2009). Collectively, these data provide *sufficient* evidence that blood Pb levels <5 µg/dL are associated with adverse effects on kidney function in adults. The NTP recognizes that blood levels measured during adulthood in this range do not preclude the possibility that an individual might have had past exposure to higher blood levels. Although several studies of groups with high occupational Pb exposure have demonstrated an association between elevated bone Pb levels and serum creatinine (e.g., Weaver *et al.* 2003, Weaver *et al.* 2005, Weaver *et al.* 2009), few studies of groups with low blood Pb levels have included bone Pb or other measures of exposure. Currently, the only data on bone Pb and kidney effects in the general population are from Tsaih *et al.* (2004) on men followed up in the Normative Aging Study for 6 years. In this study, baseline tibia Pb, but not patella Pb, was significantly associated in diabetics with baseline serum creatinine, follow-up serum creatinine, and changes in serum creatinine. Tibia Pb was also associated with a change in serum creatinine in men with hypertension. No significant associations were reported in nondiabetics or normotensives. The associations were stronger for tibia Pb than for blood Pb, measured either at baseline or follow-up.

7.3.2 Occupational Exposures

Assessment of kidney effects at higher blood Pb, such as those experienced in occupational cohorts, is beyond the scope of this evaluation but is addressed in the EPA's 2006 AQCD for Lead (U.S. EPA 2006), with an expanded discussion in EPA's current external review draft document (U.S. EPA 2012). Historically, research in occupational settings where Pb levels are higher (≥30 µg/dL) has been less consistent than findings from general population studies (U.S. EPA 2006). For example, several of these studies reported inverse associations, including higher Pb dose with lower blood urea nitrogen, serum creatinine, and/or higher creatinine clearance. These seemingly paradoxical effects compared to findings at lower Pb exposure levels may indicate different mechanisms of Pb-mediated kidney toxicity in different subgroups, namely, Pb-related hyperfiltration (U.S. EPA 2006), a condition where a sustained increase in

the kidney filtration rate can lead to kidney damage over time. However, studies published since the EPA's 2006 AQCD Lead document (U.S. EPA 2006) more consistently report worse kidney outcomes in exposed workers (Alinovi *et al.* 2005, Garcon *et al.* 2007, Lin and Tai-Yi 2007, Patil *et al.* 2007, Khan *et al.* 2008, Sun *et al.* 2008). These more recent studies in workers at higher exposure levels also lessen a previous concern that the kidney effects reported in the general populations are due to reverse causality, because no effects were observed in workers at higher exposure levels.

7.4 Susceptible Populations or Life Stages

7.4.1 Children

There is *inadequate* evidence available to address the potential association between low-level blood Pb in children <12 years of age and impaired kidney function, but *limited* evidence that blood Pb levels <5 µg/dL are associated with adverse effects on kidney function in children ≥12 years of age. Relatively few studies have addressed kidney function or serum creatinine in children compared to adults; more often early biological effect markers (EBEs) are reported (**Table 7.4**; see also Appendix A: Neurological Effects). How well EBEs predict impaired kidney function is not well established, even in adults (U.S. EPA 2006). However, Fadrowski *et al.* (2010) analyzed data from 769 children and young adults 12-20 years of age in NHANES 1988-1994 and reported a reduced mean decrease in eGFR (based on cystatin C) of 2.9 mL/min/1.73 m^2 (95% CI: -0.7, -5.0) per doubling of blood Pb in the fully adjusted model. When the data were analyzed based on categories of Pb exposure, the mean difference in eGFR was significantly reduced for the highest group (quartile 4, >2.9 µg/dL) compared to the lowest group (quartile 1, <1 µg/dL), with a mean decrease in eGFR of 6.6 mL/min/1.73 m^2 (95% CI: 0.7, 12.6), and the trend of greater decreases with higher Pb levels was significant across exposure categories (p-trend=0.009). This cross-sectional study presents the strongest indication to date for an association between low-level Pb and impaired kidney function in children and is restricted to children ≥12 years of age. It is unknown from the analysis reported in Fadrowski *et al.* (2010) whether or not the effect is driven by the older children in the study (i.e., whether decreased eGFR in children ≥17 years of age was responsible for the significant decline in the entire group from 12 to 20 years of age). The conclusion of *limited* evidence that blood Pb levels <5 µg/dL are associated with adverse effects on kidney function in children ≥12 years of age is based mainly on the Fadrowski *et al.* (2010) study, with support provided by the consistency of the data with effects observed at similar levels in adults. These findings are also consistent with reduced eGFR reported in adults (Muntner *et al.* 2003, Akesson *et al.* 2005, Muntner *et al.* 2005, Navas-Acien *et al.* 2009). This conclusion is supported by the increased serum levels of cystatin C reported in children in Belgium (Staessen *et al.* 2001), a measure that is becoming more widely accepted and used.

The existing literature does not permit a determination on relative sensitivity in children and adults. One challenge in interpreting the data for kidney effects in children is the potential for

Table 7.4: Studies of kidney outcomes in children				
Relevance to conclusions	**Study Description**	**Study Design**	**Key Kidney Findings**	**Reference**
Effect	769 children and young adults age 12-20 in NHANES 1988-1994	Cross-sectional	Reduced GFR associated with blood Pb of >2.9 µg/dL versus <1 µg/dL (β (95% CI)=-6.6 (-0.7, -12.6))	Fadrowski (2010)
Effect	Children age 17 living near industrial areas in Belgium; n=100 Pb exposed and 100 referents	Cross-sectional	Increased levels of serum cystatin C and β_2-microglobulin in children with higher blood Pb (mean, 2.7 µg/dL) compared to referents (mean, 1.5 µg/dL)	Staessen (2001)
Effect	Children 1-6 years old of workers in Pakistani Pb plants; n=123 Pb exposed and 123 referents	Cross-sectional	Increased levels of serum creatinine and urea in Pb-exposed children compared to controls (median Pb, 8.1 and 6.7 µg/dL, respectively; p≤0.01 for both measures in unadjusted analyses)	Khan (2010)
Equivocal	Children 12-15 years old in Czech Republic living near two smelters; area 1, n=91; area 2, n=53; reference site, n=51	Cross-sectional	Increased levels of urinary levels of β_2-microglobulin, Clara cell protein, and NAG in children living in area 1 (mean blood Pb: males, 10.9 µg/dL; females, 9.44 µg/dL) compared to referents (mean blood Pb: males, 8.7 µg/dL; females, 8.39 µg/dL), *but not area 2, where blood Pb levels were highest* mean blood Pb: males, 14.9 µg/dL; females, 12.9 µg/dL); increased levels of RBP in children living in both smelter areas. Significant correlation between urinary excretion and blood Pb in total group (partial r^2=0.046, regression coefficient=0.302, p=0.005)	Bernard (1995)
Equivocal	Children 10 years old in Poland living near Pb-producing factories; n=62 exposed and 50 referents	Cross-sectional	Altered urinary biomarkers (transferrin, 6-keto-$PGF_{1\alpha}$, NAG B, β_2-microglobulin, Clara cell protein, EGF, PGE_2) between 62 exposed (mean blood Pb, 13.3 µg/dL) and 50 control (mean blood Pb=3.9 µg/dL) children; *no difference in serum creatinine or serum Clara cell protein; no difference in other urine biomarkers* (e.g., fibronectin, NAG, α_1-microglobulin, RBP, total protein, and albumin laminin)	Fels (1998)
No effect	Children 8.5-12.3 years old in France living near two smelters; n=200 exposed and 200 matched referents	Cross-sectional	No difference in any kidney parameters in children living in reference (mean blood Pb: males, 3.4 µg/dL; females, 2.7 µg/dL) and polluted areas (mean blood Pb: males, 4.2 µg/dL; females, 3.7 µg/dL) (total protein, albumin, transferrin, β_2-microblobulin, RBP, brush border antigen, and NAG)	de Burbure (2003)
No effect because of direction of effect	Children 8.5-12.3 years old in Europe living near two smelters; n=364 exposed and 352 matched referents	Cross-sectional	Inverse relationship (regression coefficients) with blood Pb and serum creatinine (-0.026, p=0.007), serum cystatin C (-0.056, p=0.02), and β_2-microglobulin (-0.095, p=0.01) in children living near smelters (mean Pb: males, 4.2; females, 3.6 µg/dL) compared to controls (mean Pb: males, 3.4; females, 2.8 µg/dL)	de Burbure (2006)

Abbreviations: CI, confidence interval; EGF, epidermal growth factor; GFR, glomerular filtration rate; NAG, N-acetyl-β-D-glucosaminidase; NHANES, National Health and Nutrition Examination Survey; PGE_2, prostaglandin E_2; $PGF_{1\alpha}$, prostaglandin $F_{1\alpha}$; RBP, retinol-binding protein.

renal complications to be asymptomatic and may not become detectable until many years after exposure. This relatively slow response to Pb is supported by the animal data in which male rats exposed to Pb-acetate in drinking water did not display decreased GFR until 6 or 12 months of exposure (e.g., Khalil-Manesh *et al.* 1992a, Khalil-Manesh *et al.* 1992b, Khalil-Manesh *et al.* 1993). Young children with an age less than the latent period of clinically detectable kidney disease would not be expected to show Pb-related kidney effects. In addition, glomerular hyperfiltration during early stages of Pb nephropathy (as has been observed in rats after 3 months of exposure (Khalil-Manesh *et al.* 1992b)) would also mask the effect of Pb exposure on kidney function in young children (see also discussion of de Burbure *et al.* 2006 below). The lack of clear evidence of adverse effects of blood Pb on children <12 years of age does not exclude the potential role of Pb exposure on development of Pb-related kidney effects later in life.

The studies of serum creatinine and other blood or urine biomarkers present less indication of an effect of Pb on kidney function in children (**Table 7.4**). De Burbure *et al.* (2006) reported an association between higher blood Pb and lower serum creatinine (adjusted regression coefficient=-0.026, p=0.007); however, the opposite direction of effect, an increase in serum creatinine, would be considered to indicate decreased kidney function. Alternatively, the de Burbure *et al.* (2006) finding may be consistent with hyperfiltration. The data in children may be inconsistent because of a lack of reliable measures in children, differential effects by age (hyperfiltration vs. decreased clearance), or other factors. An earlier study found no difference in mean serum creatinine between 62 exposed (mean blood Pb, 13.3 µg/dL) and 50 control (mean blood Pb, 3.9 µg/dL) Polish children (Fels *et al.* 1998). Khan *et al.* (2010) reported a correlation of blood Pb levels with serum creatinine (r=0.13; p=0.05) in a study in 1- to 6-year-old children of workers in Pakistani Pb smelters and battery recycling plants; blood Pb, serum creatinine, and urea were higher in the children of workers (n=123 exposed) compared to controls (n=123; medians=8.1 and 6.7 µg/dL; 56 and 52 µmol/L; and 4.5 and 4.3 mmol/L, respectively; p≤ 0.01 for all in unadjusted analyses). Two studies assessed associations between blood Pb and serum cystatin C and reported opposite findings (Staessen *et al.* 2001, de Burbure *et al.* 2006). Staessen *et al.* (2001) measured higher levels of serum cystatin C in 17-year-old Belgian children living in a chemical industrial region (mean blood Pb, 2.7 µg/dL) compared to the reference group (mean blood Pb, 1.5 µg/dL). de Burbure *et al.* (2006) found an inverse association with blood Pb in 300-600 European children 8.5-12.3 years of age (adjusted regression coefficient=-0.056, p=0.02). The same pattern of opposite direction was observed for β_2-microglobulin in these two studies. Findings on urine biomarkers are also inconsistent and difficult to interpret (Bernard *et al.* 1995, Fels *et al.* 1998, de Burbure *et al.* 2003)(**Table 7.4**). For example, Bernard *et al.* (1995) found higher urinary levels of β_2-microglobulin, Clara cell protein, and N-acetyl-β-D-glucosaminidase (NAG) in children living in one "polluted" region compared to children living the reference area, but not in another "polluted" region where average blood Pb levels were the highest. The levels of blood Pb in the comparison groups are another factor to consider when interpreting the studies in children. No differences in kidney parameters were observed in de Burbure *et al.* (2003). Although the average blood Pb levels between the "exposed" and reference groups were quite similar in this study (mean blood Pb in 200 referents: males, 3.4 µg/dL; females, 2.7 µg/dL; vs. 200 children

considered exposed: males, 4.2 µg/dL; females, 3.7 µg/dL), no associations were observed when stepwise multiple regression analysis was conducted on the whole group of children as well.

Several studies have assessed the impact of higher Pb exposures during childhood on kidney measures, but these studies do not necessarily provide additional clarity (Inglis *et al.* 1978, Moel and Sachs 1992, Verberk *et al.* 1996, Coria *et al.* 2009). No nephrotoxicity was reported in a group of 77 individuals in rural Chile who were assessed 10 years after being exposed to Pb-contaminated flour as children in 1996 and subsequently treated with EDTA (Pb levels measured in 1996 ranged from 37 to 87 µg/dL) (Coria *et al.* 2009). Similarly, Moel *et al.* (1992) did not detect any significant differences between previously Pb-poisoned children and their siblings for serum creatinine, uric acid, and β_2-microglobulin, fractional excretion of β_2-microglobulin, urinary protein:creatinine ratio, and tubular reabsorption of phosphate. The study compared 62 patients at a Chicago Pb clinic, who were diagnosed and received chelation treatment between 1966 and 1972 for initial blood Pb levels >100 µg/dL, to 19 age-matched siblings whose initial blood Pb levels were <40 µg/dL. Kidney pathology was documented in certain adult survivors of untreated childhood Pb poisoning in Queensland before its removal from paint (Inglis *et al.* 1978). It is unclear whether differences in outcomes between those studies can be attributed to use of EDTA in Coria *et al.* (2009) and Moel *et al.* (1992); an elevated risk of low IQ was reported in that study. An impact on IQ but not on the kidney in that study may also suggest the kidney is less susceptible than the neurological system. Hu *et al.* (1991) reported elevated creatinine clearance rates in 21 survivors of childhood poisoning in Boston from 1930 to 1942 compared to controls matched for age, sex, race, and neighborhood (1.88 versus 1.48 mL/s per 1.73 m^2). There were no differences in serum creatinine levels between subjects and controls. The creatinine clearance finding is opposite results reported in adults in the general population discussed above but is consistent with the suggestion that Pb may induce kidney hyperfiltration in certain subpopulations. Verberk *et al.* (1996) looked at a number of kidney biomarkers in 151 children 3-6 years of age living near a Pb smelter in Baia Mare, Romania. The average Pb levels in the subgroups (based on proximity to the smelter) ranged from 34.2 to 43.8 µg/dL (Verberk *et al.* 1996). An increase in NAG per 100 µg/dL blood Pb was reported, but there were no associations with albumin, α_1-microglobulin, retinol-binding protein, or alanine aminopeptidase.

7.4.2 Hypertensives, Diabetics, and Kidney Disease Patients

The impacts of Pb on kidney function in susceptible populations, such as people with diabetes, hypertensions, or CKD, are expected to be higher (U.S. EPA 2006). This pattern is apparent in several studies that conducted subgroup analyses of NHANES data and the Normative Aging Study and found that associations were stronger in NHANES participants with hypertension (Muntner *et al.* 2003) or men in the Normative Aging Study with diabetes (Tsaih *et al.* 2004).

Follow-up studies in people with renal disease or type II diabetes also indicate worse disease progression (kidney function decline) in association with higher baseline blood Pb levels (Lin *et al.* 2003, Yu *et al.* 2004, Lin *et al.* 2006a, Lin *et al.* 2006b, U.S. EPA 2006, Weaver and Jaar 2010, U.S. EPA 2012). Although most of the patient studies are relatively small in size (ranging from

87 to 202 subjects), they are longitudinal in design, with follow-up ranging from 1.1 to 3.87 years. They provide additional support for kidney effects at low-levels of blood Pb and also support the conclusion that reverse causality is not likely to account for the effects of Pb on kidney function at low levels. In the study with the longest period of follow-up, 4 years, a 1 µg/dL higher blood Pb at baseline in patients with chronic renal insufficiency was associated with a 4.0 mL/min/1.73 m^2 reduction in eGFR (Yu *et al.* 2004). The average blood Pb level in the sample of 121 patients was 4.2 µg/dL. In order to be eligible, patients were required to have baseline EDTA-chelatable Pb below a level thought to indicate risk for Pb-related kidney toxicity. Across these studies, the decline in eGFR per 1 SD increase in Pb dose at baseline per year ranged from 0.16 (Lin *et al.* 2003) to 3.87 4.0 mL/min/1.73 m^2 (Lin *et al.* 2006b). The magnitude of decline in eGFR in the study with the lowest baseline blood Pb (2.9 µg/dL) was 1.1 per 1 SD increase in Pb dose at baseline per year (Lin *et al.* 2006b). A collection of Taiwanese patient studies included intervention arms with chelation therapy (Lin *et al.* 2003, Lin *et al.* 2006a, Lin *et al.* 2006b, Lin and Tai-Yi 2007); these studies show less kidney function decline in patients undergoing chelation therapy compared to those receiving the placebo. However, interpreting these studies is complex because of difficulty in separating outcomes that may be due to a direct beneficial effect of the chelating agent, such as via anti-oxidation, from outcomes due to Pb removal. In addition, they involve small number of patients, and the findings should be repeated in larger populations in multiple centers.

Thus, Pb-associated reduced kidney function in healthy individuals may not necessarily result in CKD, although Pb may be a risk factor for CKD in susceptible patient populations (e.g., people with kidney disease, diabetes, or obesity), in older populations, or when combined with exposure to other compounds known to cause kidney damage (e.g., cadmium, mercury, arsenic, zinc) (U.S. EPA 2006).

7.5 Conclusions

The NTP concludes that there is *sufficient* evidence that blood Pb levels <5 µg/dL are associated with adverse effects on kidney function in adults (see **Table 7.5** for complete list of kidney effects conclusions). With few exceptions, epidemiological studies of the general population reported associations between blood Pb levels <10 µg/dL and (1) increased risk of CKD and (2) decreased kidney function as measured by GFR and creatinine clearance. The NTP recognizes that blood levels in this range measured during adulthood do not preclude the possibility that an individual might have had past exposure to higher blood levels. As with other studies of health effects of Pb in adults, prospective studies in a group for which blood Pb levels remained consistently below 10 µg/dL from birth until measurement would eliminate the potential role of early-life blood Pb levels above 10 µg/dL on health effects observed in adults with concurrent blood Pb levels <10 µg/dL. The associations are typically stronger in people with hypertension or diabetes (Muntner *et al.* 2003, Tsaih *et al.* 2004). The NTP also concludes that there is *limited* evidence that blood Pb levels <5 µg/dL are associated with adverse effects on kidney function in children ≥12 years of age based on the recent cross-sectional data from the NHANES data set published by Fadrowski *et al.* (2010) and the consistency of effects with observations in adults. There is *inadequate* evidence that blood Pb levels <10 µg/dL are associated with kidney

function in children <12 years of age because of inconsistent results and studies lacking clear predictive measures of kidney function in children. The lack of consistent predictive measures of kidney function in children makes studying the effects of Pb on this life stage difficult. The NTP's conclusions of *sufficient* evidence that blood Pb levels <5 µg/dL are associated with adverse effects on kidney function in adults and *limited* evidence in children ≥12 years of age, extend the conclusions of the 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) and 2006 EPA AQCD for Lead (U.S. EPA 2006) from adults to children age 12 and older based on recent data.

Table 7.5: NTP conclusions on kidney effects of low-level Pb

Health Effect	Population	NTP Conclusions	Blood Pb Evidence	Bone Pb Evidence
Increased chronic kidney disease (CKD) and decreased glomerular filtration rate(GFR)	Adults	*Sufficient*	Yes, <5 µg/dL	Not studied
	Children ≥12 years old	*Limited*	Yes, <5 µg/dL	Not studied
	Children <12 years old	*Inadequate*	Unclear	Not studied

8.0 REPRODUCTIVE / DEVELOPMENTAL EFFECTS

8.1 Conclusions

The NTP concludes there is *sufficient* evidence that blood Pb levels <10 µg/dL are associated with adverse health effects on development in children and that blood Pb levels <5 µg/dL are associated with adverse health effects on reproduction in adult women.

Because the database of human studies on most reproductive endpoints is limited to occupational exposure studies, many of the available studies are for blood Pb levels >10 µg/dL. Given this fact and the focus of the original nomination on reproductive and developmental effects, higher blood Pb levels were included in the evaluation of these health effects, unlike other sections of this document. Consideration of blood Pb levels >10 µg/dL resulted in several conclusions for Pb-related reproductive effects in men but did not affect the conclusions for women or children.

Unlike the data set for most other health effects, a number of prospective studies of developmental effects include prenatal measures of exposure (either maternal blood or umbilical cord blood). Maternal blood Pb <10 µg/dL is associated with decreased head circumference in children through 4 years of age, providing evidence that prenatal exposure is associated with reduced postnatal growth in children. Conclusions on effects of prenatal exposure for outcomes evaluated as children are complicated by the high degree of correlation in childhood blood Pb levels over time, and as described below, concurrent blood Pb levels <10 µg/dL in children are also associated with reduced postnatal growth.

Children

In children, there is *sufficient* evidence that blood Pb levels <10 µg/dL are associated with delayed puberty in both boys and girls. Nine studies with mean blood Pb levels <10 µg/dL support the relationship between Pb and delayed puberty (see **Table 8.3**); although several studies report effects on puberty at blood Pb levels <5 µg/dL, there is also evidence indicating no effect of blood Pb <5 µg/dL. Therefore, there is *limited* evidence that delayed puberty is associated with blood Pb levels <5 µg/dL. There is *sufficient* evidence that decreased postnatal growth is associated with blood Pb levels <10 µg/dL in children. Epidemiological studies consistently report an inverse relationship between blood Pb levels <10 µg/dL and postnatal growth (see **Table 8.4**). Developmental effects on neurological, immunological, renal, and cardiovascular systems are not covered in this section because they are reviewed in individual chapters.

Women

In adult women, there is *sufficient* evidence that maternal blood Pb levels <5 µg/dL are associated with reduced fetal growth or lower birth weight. The association between maternal Pb exposure and reduced fetal growth is supported by several prospective studies with maternal blood Pb data during pregnancy, a large retrospective cohort of over 43,000 mother-infant pairs that reports a mean maternal blood Pb level of 2.1 µg/dL, and a number of cross-

sectional studies with maternal or umbilical cord blood Pb at delivery. Although maternal or paternal bone Pb data are not available in studies of most reproductive health outcomes, a set of studies from a single group reported that maternal bone Pb is associated with lower birth weight, birth length, and head circumference. There is *limited* evidence that maternal blood Pb levels <10 µg/dL are associated with spontaneous abortion and preterm birth or reduced gestational age. Although a number of prospective studies with maternal blood Pb levels during pregnancy and cross-sectional studies with umbilical cord blood Pb levels at delivery reported an association between prenatal blood Pb levels <10 µg/dL and preterm birth, the conclusion of *limited* evidence is based on the inconsistent results and because a retrospective study with a large cohort of over 43,000 mother-infant pairs did not find an association between maternal blood Pb levels and preterm birth. The conclusion of *limited* evidence for an association with spontaneous abortion in women is based principally on the Borja-Aburto *et al.* (1999) study, which has the strength of its prospective nested case-control design, with additional support provided by occupational studies that reported an association with Pb exposure but lack blood Pb measurements. There is *inadequate* evidence for other reproductive and effects of Pb associated with blood Pb levels <10 µg/dL in women.

Men

In adult men, there is *inadequate* evidence that blood Pb levels <10 µg/dL are associated with effects on reproduction. There is *sufficient* evidence that blood Pb levels ≥15 µg/dL are associated with adverse effects on sperm or semen in men, and *inadequate* evidence for adverse effects on sperm at lower blood Pb levels. Decreased sperm count, density, and/or concentration has been reported in multiple retrospective and cross-sectional occupational studies of men with mean blood Pb levels from 15-68 µg/dL (see **Table 8.5**). There is *sufficient* evidence that paternal blood Pb levels ≥20 µg/dL are associated with delayed conception time and *limited* evidence that blood Pb levels ≥10 µg/dL in men are associated with other measures of reduced fertility. Four studies reported increased time to pregnancy in women whose male partners had blood Pb levels of 20-40 µg/dL. A single retrospective occupational study reported increased risk of infertility among men with blood Pb levels ≥10 µg/dL, and the continuity of these data with effects on time to pregnancy supports a conclusion of *limited* evidence that blood Pb levels ≥10 µg/dL in men are associated with other measures of reduced fertility. There is *limited* evidence that paternal blood Pb >31 µg/dL is associated with spontaneous abortion. The conclusion of *limited* evidence that spontaneous abortion is associated with paternal exposure is based mainly on the retrospective nested case-control study by Lindbohm *et al.* (1991a) in men, with additional support provided by occupational studies that reported an association with Pb exposure but lack blood Pb measurements.

8.2 How Conclusions Were Reached

Conclusions in the NTP's evaluation of Pb-related reproductive and developmental effects in humans associated with low-level Pb are derived by evaluating the data from epidemiological studies with a focus on blood Pb levels <10 µg/dL. Because the database of human studies on

most reproductive endpoints is limited to occupational exposure studies, many of the available studies are for blood Pb levels >10 µg/dL. Unlike other sections of this document, reproductive effects of these higher blood Pb levels were included in the evaluation because the data set of human studies on potential reproductive effects associated with lower blood Pb levels is limited. Major endpoints considered as potential indicators of effects of Pb on reproduction and development are listed and briefly described in **Section 8.2.1**. This document is not a review of the reproductive system or reproductive and developmental toxicity, and the reader is directed to published reviews for additional background. Key data and principal studies considered in developing the NTP conclusions are discussed in detail in **Section 8.3 Evidence for Pb-related Effects on Reproductive and Developmental Outcomes**. The discussion of each effect begins with a statement of the NTP's conclusion that the specific effect is associated with a blood Pb level <10 µg/dL or <5 µg/dL and the age group in which it is identified (childhood or adulthood), as well as the timing of exposure associated with the effect (prenatal, childhood, concurrent) when available. The discussion also highlights the extent to which experimental animal data support the association between Pb exposure and reproductive effects. Although the information necessary to support the NTP conclusions is presented in **Section 8.3**, the complete data set of human studies considered for evaluation of reproductive and developmental effects with low-level Pb is included in Appendix E: Reproductive and Developmental Effects, and individual studies are abstracted for further reference. The NTP made extensive use of recent government evaluations of health effects of Pb in the current assessment, and the relevant conclusions of the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's 2007 Toxicological Profile for Lead (ATSDR 2007) are briefly described in **Section 8.2.2** below.

8.2.1 Principal Measures of Reproductive and Developmental Effects

Table 8.1 lists a number of key reproductive and developmental endpoints commonly evaluated in epidemiological studies. The data available to evaluate each of the major effects are discussed in separate subheadings under **Section 8.3 Evidence for Pb-related Effects on Reproductive and Developmental Outcomes** below.

Table 8.1: Major reproductive/developmental effects considered

Effect	Description
Delayed puberty	Delay in measures of puberty (e.g., Tanner staging of genitalia, pubic hair, and breast development)
Postnatal growth	Slower growth (as indicated by height, head circumference, etc., for age)
Sperm parameters	Numerous sperm or semen measures (sperm count, motility, morphology, etc.)
Conception	Greater time to pregnancy or lower fecundity
Pregnancy loss	Spontaneous abortion (fetal loss <20 weeks gestation), stillbirth (fetal loss ≥20 weeks)
Gestation length	Shorter length of gestation (as a continuous measure), preterm birth (<37 weeks)
Fetal growth	Lower birth weight, often adjusted for gestational age
Birth defects	Congenital malformations

8.2.2 Principal Conclusions from 2006 EPA and 2007 ATSDR Pb Documents:

The EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's 2007 Toxicological Profile for Lead (ATSDR 2007) both concluded that there is evidence for reproductive effects in males at high blood Pb levels (30-40 µg/dL in ATSDR, 2007 and 45 µg/dL in U.S. EPA, 2006) and suggest that more research is needed to determine if effects occur at lower blood Pb levels (see **Table 8.2** for principal conclusions and original documents for complete conclusions). Although the 2006 EPA AQCD for Lead (U.S. EPA 2006) cited the Borja-Aburto *et al.* study (1999) as a well-conducted prospective case-control study supporting a significant relationship between maternal blood Pb level at 10 µg/dL to 12 µg/dL and spontaneous abortion, the EPA concluded that, collectively, there is little evidence to support an association between maternal or paternal Pb exposure and incidence of spontaneous abortion. The 2006 EPA AQCD for Lead (U.S. EPA 2006) concluded that the data are inadequate to evaluate reproductive effects in females, and studies of developmental effects suggest at most a small association between Pb exposure and preterm birth, congenital abnormalities, birth weight, or fetal growth. The EPA is in the process of revising the AQCD, and the conclusions of the external draft (U.S. EPA 2012) are largely in line with the 2006 AQCD for Lead (U.S. EPA 2006), plus additional review of the evidence for delayed pubertal onset.

Table 8.2: Main conclusions for reproductive / developmental effects in the 2006 EPA AQCD for Lead and the 2007 ATSDR Toxicological Profile for Lead

"The epidemiologic evidence suggests small associations between exposure to Pb and male reproductive outcomes, including perturbed semen quality and increased time to pregnancy. These associations appear at blood Pb levels >45 µg/dL, as most studies have only considered exposure in the occupational setting. There are no adequate data to evaluate associations between Pb exposure and female fertility." (U.S. EPA 2006, pg 6-271)
"Studies of children also have shown associations between PbB [blood Pb level] and growth, delayed sexual maturation in girls, and decreased erythropoietin production. Some studies of humans occupationally or environmentally exposed to Pb have observed associations between PbB and abortion and preterm delivery in women and alterations in sperm and decreased fertility in men. On the other hand, there are several studies that found no significant association between Pb exposure and these end points. At least for the effects in males, the threshold PbB appears to be in the range of 30-40 µg/dL." (ATSDR 2007, pg 23)

The NTP considered the conclusions and data summaries from the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's 2007 Toxicological Profile for Lead (ATSDR 2007). In general, the NTP concurred with the conclusions and agreed that the data support them. Differences from the ATSDR and EPA documents are identified for specific endpoints in this document.

8.3 Evidence for Pb-related Effects on Reproductive and Developmental Outcomes

8.3.1 Delayed Puberty

There is *sufficient* evidence that blood Pb levels <10 µg/dL are associated with a delay in sexual maturation or puberty onset in children 8-17 years of age (see **Table 8.3** and the Puberty section of Appendix E: Reproductive and Developmental Effects). An inverse association

between blood Pb level and markers of sexual maturation has been reported in eight cross-sectional studies and a single prospective study involving children with blood Pb levels from 10 to <1 μg/dL from seven different populations in North America, Europe, and Africa. Pb-related developmental delay in a several biological markers of puberty (e.g., age at menarche and Tanner developmental staging of breasts) have been reported in cross-sectional studies of girls, although there is no single measure that is consistently associated with blood Pb levels in all analyses. For boys, a Pb-related decrease in testicular volume was observed in all four publications, suggesting that testicular volume may be a reliable indicator of the effects of Pb on puberty in boys. The reported delay in sexual maturation with increasing blood Pb was significant across multiple studies, in various endpoints, and from different groups in analyses that adjusted for factors known to effect puberty such as race, BMI, and socioeconomic status. A conclusion of *limited* evidence that delayed puberty is associated with blood Pb levels <5 μg/dL is based on the four studies (three in girls and one in boys) that report delay in markers of puberty associated with blood Pb levels <5 μg/dL and the lack of association with blood Pb among girls in the Wolff *et al.* (2008) study with a median blood Pb level of 2 μg/dL.

Three cross-sectional studies using the NHANES III data set reported delayed puberty onset in girls. Wu *et al.* (2003) reported delayed Tanner pubic hair stage and age at menarche in girls 8-16 years of age with blood Pb levels ≥2 μg/dL, with no effect on developmental stage of breasts. In a separate analysis divided by race and ethnicity, Selevan *et al.* (2003) reported an association between blood Pb >3 μg/dL and delayed puberty onset in African American and Mexican American girls as determined by Tanner pubic hair and breast stages. Age at menarche was also delayed at blood Pb levels >3 μg/dL in African American girls; however, there was no Pb-related effect on puberty in non-Hispanic whites. Gollenberg *et al.* (2010) reported that girls with blood Pb levels ≥5 μg/dL were less likely to have levels of inhibin B of >35 pg/mL, a level that the authors suggest is associated with pubic hair and breast development. A similar delay in puberty onset indicated by age at menarche was reported in 13-year-old girls in South Africa (Naicker *et al.* 2010) and 10- to 17-year-old girls from the Mohawk Nation (Denham *et al.* 2005). Naicker *et al.* (2010) also found an association with blood Pb ≥5 μg/dL and delays pubic hair and breast development stage. In contrast, Tomoum *et al.* (2010) reported decreased Tanner breast developmental stage and no effect on pubic hair in girls 10-13 years of age with blood Pb>10 μg/dL in Egypt. Staessen *et al.* (2001) reported significant (p=0.04) differences in breast development in 17-year-old girls among three groups: two study groups with blood Pb levels of 1.8 and 2.7 μg/dL and a reference group with blood Pb of 1.5 μg/dL. However, a direct comparison of breast stage by blood Pb levels was not reported, and the delay in breast stage was significant only when comparing the study group with mean blood Pb of 1.8 μg/dL to the reference group. One of the eight studies reporting data on girls did not detect a delay in any marker of puberty onset associated with blood Pb; mean blood Pb was 2 μg/dL in the 9-year-old girls from New York in that study (Wolff *et al.* 2008).

Table 8.3: Studies of biomarkers of puberty associated with low-level Pb exposure used to develop conclusions				
Relevance to Conclusions	**Study Description**	**Study Design**	**Key Reproductive/Developmental Findings**	**Reference**
Chapaevsk, Russia		Prospective	Delayed puberty onset in boys with blood Pb ≥5 µg/dL. Puberty measures differed by testicular volume and Tanner genital and pubic hair staging.	Williams (2010) *(same population as Hauser)*
Effect	Boys 11-12; n=481			
	Boys 8-9; n=489	Cross-sectional	Delayed puberty onset in boys with blood Pb ≥5 µg/dL. Puberty determined by Tanner genital staging and testicular volume; effects not significant for pubic hair staging.	Hauser (2008)
NHANES III		Cross-sectional	Delayed puberty onset in girls with blood Pb ≥2 µg/dL compared to those with blood Pb <2 µg/dL. Puberty differed by Tanner pubic hair developmental stage and age at menarche; no effect on developmental stage of breasts.	Wu (2003) *(population overlap with Gollenberg, Selevan)*
Effect	Girls 8-16 years of age; n=1,235			
	Girls 8-11 years old; n=705	Cross-sectional	Girls with higher blood Pb (blood Pb ≥5 µg/dL compared with those <1 µg/dL) had lower likelihood of having inhibin B levels >35 pg/mL, a level the authors report to be associated with puberty and development of pubic hair and breasts.	Gollenberg (2010)
	Girls 8-16 years old; n=600-805 per race/ethnicity	Cross-sectional	Delayed puberty onset in African American girls (age at menarche, Tanner breast and pubic hair developmental stage) and Mexican American girls (breast and pubic hair stage) at blood Pb >3 µg/dL compared to blood Pb <1 µg/dL; not in non-Hispanic whites.	Selevan (2003)
Effect	Girls 13 years old in South Africa; n=682-712, by endpoint	Cross-sectional	Delayed puberty onset (determined by Tanner pubic hair and breast developmental stage and age at menarche) in girls with blood Pb ≥5 µg/dL, and significant association with blood Pb by trend analysis across for stage or age at menarche.	Naicker (2010)
Effect	Children aged 17 in Belgium; n=100 Pb and 100 referent	Cross-sectional	Testicular volume was lower in boys living in areas with higher blood Pb (1.8-2.7 µg/dL) compared to referents (1.5 µg/dL). Genital and breast stage did not differ consistently and significantly between referent and exposed; comparison by blood Pb not reported.	Staessen (2001)
Effect	Girls aged 10-17 in Akwesasne Mohawk Nation; n=138	Cross-sectional	Delayed puberty onset (age at menarche) in girls with blood Pb above mean (≥0.49 µg/dL) and a predicted delay in age at menarche of 10 months with blood Pb above median (1.2 µg/dL).	Denham (2005)
No effect	Girls 9 years old in New York; n=139	Cross-sectional	Blood Pb had no effect on puberty onset in girls (by breast and pubic hair stage) with median blood Pb level of 2 µg/dL.	Wolff (2008)
Effect	Children 10-13 years old in Egypt; n=41	Cross-sectional	Delayed puberty onset in boys and girls with blood Pb ≥10 µg/dL. Puberty measures differed for testes size, Tanner pubic hair and penile stage in boys, and Tanner developmental stage of breasts in girls, but not pubic hair in girls.	Tomoum (2010)

Epidemiological studies of Pb exposure and puberty are listed by decreasing cohort size and grouped together for overlapping or shared study groups.
Abbreviations: NHANES: National Health and Nutrition Examination Survey.

All four of the studies addressing boys report Pb-associated decreases in testicular volume or Tanner genital or pubic hair staging (Staessen *et al.* 2001, Hauser *et al.* 2008, Tomoum *et al.* 2010, Williams *et al.* 2010). In a paired cross-sectional and follow-up prospective study, blood Pb ≥5 µg/dL was associated with delayed puberty that was significant by Tanner genital staging ($p<0.05$) and reduced testicular volume ($p\leq0.05$) in Russian boys when they were 8-9 years of age and again at 11-12 years of age (Hauser *et al.* 2008, Williams *et al.* 2010). Testicular volume was significantly lower in 17-year-old boys living in areas with higher blood levels (1.8-2.7 µg/dL) compared to a reference group (1.5 µg/dL), although the authors do not include a direct comparison of testicular volume by blood Pb levels (Staessen *et al.* 2001). Blood Pb ≥10 µg/dL was associated with decreased testes size and developmental delay in Tanner pubic hair and penile stages in 10- to 13-year-old boys in Egypt (Tomoum *et al.* 2010).

Summary of support for conclusions

Animal data supports a Pb-associated developmental delay in sexual maturation, indicated by biomarkers of puberty such as reduced prostate weight and delay in vaginal opening, at high blood Pb levels in some studies (i.e., 40 to >300 µg/dL) in Fisher and Sprague Dawley rats and at blood Pb levels similar to the human studies (i.e., 3-13 µg/dL) in Swiss mice (see U.S. EPA 2006 for recent reviews of the animal data, ATSDR 2007). The mouse data from Iavicoli *et al.* (2004) are interesting because the exposure that resulted in blood Pb from 3 to 13 µg/dL is associated with delayed puberty in female mice similar to the human data, whereas blood Pb <3 µg/dL was associated with accelerated time to puberty in mice, which suggests the possibility of a different mechanism of action in mice at blood Pb levels <3 µg/dL. The human data supporting Pb-associated delay in puberty include the one prospective study in boys discussed above, with the rest being cross-sectional studies. The determination of causation from cross-sectional studies has a serious limitation because cross-sectional studies rely on concurrent blood Pb measurements and provide no information on cumulative Pb or Pb exposure at earlier time points that may be critical for sexual maturation. However, the consistency of effects across studies, among multiple groups, and in multiple measures of puberty in both males and females lends weight to the evidence for developmental delay in puberty at blood Pb concentrations from 10 to <1 µg/dL. The conclusion of *sufficient* evidence for developmental delay in puberty in children at blood Pb levels <10 µg/dL is based on the prospective study and eight cross-sectional studies that report effects above the mean blood Pb level (0.49 µg/dL to 5 µg/dL) of the group under study. A lower effect level and the conclusion of *limited* evidence for delayed puberty at blood Pb <5 µg/dL is supported by two of the studies (Selevan *et al.* 2003, Wu *et al.* 2003) that use the NHANES III data to examine puberty onset in girls from 8-16 years of age, the Denham *et al.* (2005) study of 10- to 17-year-old girls in the Mohawk Nation, and the Staessen *et al.* (2003) study reporting lower testicular volume in boys. The Tomoum *et al.* (2010) study provides additional support but reported delay in measures of puberty in boys and girls at blood Pb levels ≥10 µg/dL. The NTP's conclusions for *sufficient* evidence for delay in sexual maturation in boys and girls at blood Pb levels <10 µg/dL and *limited* evidence at blood Pb <5 µg/dL expands the conclusion from ATSDR's 2007 Toxicological Profile for Lead (ATSDR 2007), which was limited to girls at blood Pb levels <10 µg/dL; the EPA's 2006 AQCD for Lead (U.S. EPA 2006) did not present specific conclusions on sexual maturation.

8.3.2 Postnatal Growth

There is *limited* evidence that maternal Pb <10 µg/dL is associated with decreased head circumference in children up to 4 years of age and *sufficient* evidence that concurrent blood Pb <10 µg/dL in children is associated with decreased postnatal growth. Prospective studies in two groups (Rothenberg *et al.* 1993, Rothenberg *et al.* 1999, Schell *et al.* 2009) report an inverse association between maternal blood Pb and head circumference, and one study reports the lack of an association between maternal blood Pb and height or weight through 10 years of age (Lamb *et al.* 2008). The data from prospective studies (see **Table 8.4** and Postnatal Growth section of Appendix E: Reproductive and Developmental Effects) in two groups support an association between higher blood Pb levels in children and lower subsequent growth (Greene and Ernhart 1991, Rothenberg *et al.* 1993, Rothenberg *et al.* 1999). Numerous cross-sectional studies report an association between higher blood Pb in children and smaller head circumference, height, or other indicators of growth (e.g., weight, chest circumference, etc.). The clear majority of cross-sectional studies, including studies with large numbers of subjects, such as the NHANES data sets, demonstrate an association between higher concurrent blood Pb (means from 2 to 15 µg/dL) and lower height or other indicators of postnatal growth in children from 1 to 16 years of age. This strong evidence for an association between growth and concurrent blood Pb is based on exposure data that is largely outside the relevant time window—the relevant timing of Pb exposure to effect growth is during or before the growth that results in differences in height. Therefore, the conclusion of *sufficient* evidence that blood Pb <10 µg/dL in children is associated with decreased growth is based on the combination of strong support from the cross-sectional studies and the additional support from the three prospective studies that evaluate blood Pb levels in children on subsequent growth rather than current height.

Several prospective studies support an inverse association between maternal Pb <10 µg/dL and postnatal growth as indicated by head circumference, but the relationship is less clear for height. Higher maternal blood Pb at 36 weeks of gestation was associated with smaller head circumference up to 4 years of age in children in the Mexico City Prospective Study (Rothenberg *et al.* 1993, Rothenberg *et al.* 1999). Schell *et al.* (2009) also report an effect of maternal blood Pb on head circumference, but not on height or weight, in 6- to 12-month-olds in Albany, NY. Maternal blood Pb was not related to height or weight in children from 1 to 10 years of age in the Yugoslavia Prospective Study (Lamb *et al.* 2008). Data from the Cincinnati Pb study support a combined effect of maternal blood Pb as well as concurrent blood Pb levels in the children; height at 15 months was decreased only in children with blood Pb >3.4 µg/dL that also experienced maternal blood Pb >7.7 µg/dL (Shukla *et al.* 1989). A subsequent study supported the combined effect of blood Pb at 3-15 months of age and concurrent blood Pb level for height at 33 months (Shukla *et al.* 1991). In children from the Mexico City Prospective Study, infant blood Pb at 1 year of age was inversely associated with head circumference up to 4 years of age (Rothenberg *et al.* 1993, Rothenberg *et al.* 1999). Greene *et al.* (1991) reported that blood Pb in children at 6 months of age was related to subsequent growth at borderline statistical

Table 8.4: Studies of postnatal growth associated with low-level Pb exposure used to develop conclusions				
Relevance to Conclusions	**Study Description**	**Study Design**	**Key Reproductive/Developmental Findings**	**Reference**
Cincinnati Pb study		Prospective	Concurrent blood Pb (>3.4 µg/dL) in children was inversely associated with growth rate (length) at 15 months of age in children of mothers with Pb >7.7 µg/dL.	Shukla (1989)*(same study population as Shukla, 1991)*
Effect	Children 15 months of age in; n= 260			
	Children ≤33 months of age; n=235	Prospective	Current blood Pb was inversely associated with length at 33 months of age in children with higher blood Pb (>10.8 µg/dL) from 3 to 15 months of age.	Shukla (1991)
Albany Pregnancy Infancy Pb Study		Prospective	Maternal blood Pb (≥3 µg/dL) was inversely associated with infant head circumference at 6 and 12 months, but not with length or weight.	Schell (2009)
Effect	Children 0.5-1 years old in Albany; n=211			
Cleveland Pb study		Prospective	Blood Pb at 6 months of age (10 µg/dL) was related to subsequent head circumference (p=0.05) and marginally related to subsequent length (p=0.06) and weight (p=0.08); blood Pb at 1-4 years old was not related to weight, length, head circumference at 4 years old.	Greene (1991)
Effect	Children <5 years; n= 151-185 per sample			
Mexico City Prospective Study		Prospective	Maternal blood Pb at 36 weeks (median, <10 µg/dL) was inversely associated with infant head circumference at 6 and 18 months. Infant blood Pb (1 year) was inversely associated with head circumference at 36 months.	Rothenberg (1993) *(same population as Rothenberg, 1999)*
Effect	Children 0.5-1 years; n=50-111 per sample			
	Children 0.5-4 year; n =119-199 per sample	Prospective	Maternal (36 weeks) and infant (1 year) blood Pb (median, <10 µg/dL) were inversely associated with infant head circumference at later ages, up to 4 years of age.	Rothenberg (1999)
Yugoslavia Prospective Study		Prospective	Maternal blood Pb was not correlated to height or weight in children from 1 to 10 years of age.	Lamb (2008)
No effect	Children birth, 1, 4, 6, and 10 years; n=309			
Effect	Children at 4 years of age; n=156-175	Cross-sectional	Concurrent blood Pb (<15 µg/dL) was inversely associated with height in Pristina, but blood Pb (20-40 µg/dL) was not related to height in Titova-Mitrovica, a Pb smelter town.	Factor-Litvak (1999)
NHANES III		Cross-sectional	Concurrent blood Pb (mean, 3.6 µg/dL) was inversely associated with height and head circumference but not with weight.	Ballew (1999)*(same study population as Selevan)*
Effect	Children 1-7 years old; n=4,391			
	Girls 8-16 years of age; n=600-805 per race/ethnicity	Cross-sectional	Concurrent blood Pb ≥3 µg/dL was associated with decreased height compared to individuals with a blood Pb of 1 µg/dL but not with weight.	Selevan (2003)
NHANES II		Cross-sectional	Concurrent blood Pb (range, 5-35 µg/dL) was inversely associated with height, weight, and chest circumference.	Schwartz (1986)*(same study population as Frisancho)*
Effect	Children 0.5-7 years of age; n=2695			

Table 8.4: Studies of postnatal growth associated with low-level Pb exposure used to develop conclusions				
Relevance to Conclusions	**Study Description**	**Study Design**	**Key Reproductive/Developmental Findings**	**Reference**
	Mexican-Americans age 5-12; n=1,454	Cross-sectional	Concurrent blood Pb (mean: boys, 10.6 µg/dL; girls, 9.3 µg/dL) was inversely associated with height.	Frisancho (1991)
Effect	Children aged 7-15 in Poland; n=899	Cross-sectional	Concurrent blood Pb (mean, 7.7 µg/dL) was inversely associated with height, leg length, arm length in both, trunk length in boys, weight in girls; *not weight in boys, trunk in girls.*	Ignasiak (2006)
Effect	Children aged 2-12 in Dallas; n=764 (1980s: n=404; 2002: n=390)	Cross-sectional	Concurrent blood Pb was inversely associated with height, weight, and head circumference. The height-Pb relationship was not statistically different between children in 1980s (mean=24.8 µg/dL) or 2002 (mean= 1.8 µg/dL).	Little (2009)
Effect	Children 7 years old; n=602 in Mexico	Cross-sectional	Concurrent blood Pb (11.5 µg/dL) was inversely associated with height and *positively associated with head circumference.*	Kordas (2004)
Effect	Children aged 6-9 in Greece; n=522	Cross-sectional	Concurrent blood Pb (mean, 12.3 µg/dL) was inversely associated with height, head circumference, and chest circumference.	Kafourou (1997)
Effect	Boys 8-9 in Russia; n=489	Cross-sectional	Concurrent blood Pb (median, 3 µg/dL) was inversely related to height but *not with weight or BMI.*	Hauser (2008)
Effect	Children 11-13 years old in Italy; n=418	Cross-sectional	Concurrent blood Pb inversely associated with height, weight in 13-year-old boys (mean, 8.5 µg/dL) and height in 12-year-old girls (mean, 7 µg/dL), *not children of other ages*.	Vivoli (1993)
Effect	12-month-old infants in Mexico City; n=329	Prospective & cross-sectional	Infant blood Pb (6.8 µg/dL) at 1 month and maternal bone Pb (tibia 10.1 µg/g) were inversely related to infant weight and/or weight gain through 12 months of age.	Sanin (2001)
No effect	Children 6-9 years old in Malaysia; n=268	Cross-sectional	Concurrent blood Pb (mean, 3.75 µg/dL) was not correlated to height, weight, or arm circumference for age.	Zailina (2008)
Effect	Children aged 7 and 20 in USA; n=236	Prospective & cross-sectional	Dentin Pb level of teeth lost before age 7 was inversely associated with BMI at age 7 and BMI at age 20, *not weight or height.* No association between growth & bone Pb at age 20	Kim (1995)
Effect	Children 1-10 years old in Dallas; n=139	Cross-sectional	Concurrent blood Pb was inversely associated with height, weight, and head circumference.	Little (1990)
Effect	Children 5-13 years old in Korea; n=108	Cross-sectional	Concurrent blood Pb (mean, 2.4 µg/dL) was inversely associated with height and arm length but *not with weight or BMI.*	Min (2008)
No effect	Children 10-13 years old in Egypt; n=41	Cross-sectional	Mean height and weight did not differ as a percentage of median for age and sex for individual above and below blood Pb of 10 µg/dL in children with mean Pb of 9.46 µg/dL.	Tomoum (2010)
Effect	Children age 18-36 mo. in Omaha; n=21	Cross-sectional	Concurrent blood Pb (mean, 6.4 µg/dL) was inversely associated with head circumference.	Stanek (1998)

Epidemiological studies of Pb exposure and growth are listed by decreasing cohort size and grouped together for overlapping or shared study groups.
Abbreviations: BMI, body mass index; NHANES, National Health and Nutrition Examination Survey.

significance for head circumference (p=0.05), length (p=0.06), and weight (p=0.08) in children in the Cleveland Lead Study (n=151-185 per sample).

The clear majority of cross-sectional studies support an inverse association between concurrent blood Pb (with mean levels of 2-15 µg/dL and higher) and height. Additional measures of growth such as head circumference, arm or leg length, and weight were also related to blood Pb in some studies, but these endpoints were less widely reported, and weight is less consistently associated with blood Pb. Data from NHANES II support an inverse association between concurrent blood Pb from 5 to 35 µg/dL and height, weight, and chest circumference (Schwartz *et al.* 1986, Frisancho and Ryan 1991). The inverse relationship between height (but not weight) and blood Pb was further supported by studies using data from NHANES III on children 1-7 years of age with mean blood Pb of 4 µg/dL (Ballew *et al.* 1999) and in girls 8-16 years of age with blood Pb ≥3 µg/dL (Selevan *et al.* 2003) compared to girls with blood Pb of 1 µg/dL. Other large cross-sectional studies have reported a similar inverse relationship between blood Pb and markers of growth: height and leg and arm length in Polish children 7-15 years of age (8 µg/dL mean blood Pb and n=899) (Ignasiak *et al.* 2006); height, weight, and head circumference in Children 2-12 years of age in Dallas (25 µg/dL mean blood Pb and n=764; 2 µg/dL mean blood Pb and n=390) (Little *et al.* 2009); height in 7-year-olds in Mexico (12 µg/dL mean blood Pb; n=602) (Kordas *et al.* 2004); height, head circumference, and chest circumference in children 6-9 years of age in Greece (12 µg/dL mean blood Pb; n=522) (Kafourou *et al.* 1997); and height in boys 8 and 9 years of age in Russia (3 µg/dL median blood Pb; n=489) (Hauser *et al.* 2008). None of the studies that support an effect level <5 µg/dL controlled for parental height, which may relate to the cross-sectional nature of the studies. However, parental height is considered in prospective studies as well as a few cross-sectional analyses that support an association with blood Pb levels <10 µg/dL (Shukla *et al.* 1989, Greene and Ernhart 1991, Shukla *et al.* 1991, Vivoli *et al.* 1993, Kafourou *et al.* 1997, Sanin *et al.* 2001, Schell *et al.* 2009).

There are also a number of smaller cross-sectional studies with sample sizes ranging from 330 to 21 that support an association between lower height and higher concurrent blood Pb levels (see **Table 8.4** and Postnatal Growth section of Appendix E: Reproductive and Developmental Effects). The data are not completely consistent, because Zailina *et al.* (2008) did not find a correlation between blood Pb in a study of 269 children in Malaysia in the relative height for age, and Tomoum *et al.* (2010) did not find a difference between individuals above and below 10 µg/dL in a study of 41 children 12 years of age in Egypt in the mean height as a percentage of median height. Although the finding of an association in a study with a small sample size suggests a larger magnitude of effect, it is less informative when studies with a small sample size such as the data from Egypt, fail to find an effect. In the Yugoslavia Prospective Study, higher blood Pb in 4-year-olds was associated with lower height in Pristina at blood Pb levels <15 µg/dL, but blood Pb levels from 20 to 40 µg/dL in Titova-Mitrovica (a Pb smelter town) were not associated with height (Factor-Litvak *et al.* 1999). Although Vivoli *et al.* (1993) reported an association between higher blood Pb and lower height in girls from 11 to 13 years of age, 12-year-old girls, and 13-year-old boys, the association was not significant for all age

groups (i.e., not for all boys combined, 11-year-old boys, 11-year-old girls, 12-year-old boys, or 13-year-old girls).

Few studies of growth include measures of exposure other than blood Pb data. Kim *et al.* (1995) reported an association between higher dentin Pb levels in teeth lost before 7 years of age and lower BMI at 20 years of age; however, neither dentin Pb at 7 years of age nor concurrent bone at 20 years of age was related to height or weight in the 20-year-olds. Sanin *et al.* (2001) reported an association between either maternal bone Pb or infant blood Pb and lower weight and less weight gain through 12 months of age, but the study did not report data on height.

Summary of support for conclusions

Animal data support a decrease in postnatal growth rate associated with prenatal and developmental Pb exposure at high blood Pb levels in some studies (i.e., 40-100µg/dL Sprague Dawley rats; see U.S. EPA 2006 for recent reviews of the animal data , ATSDR 2007). The human data include multiple prospective studies and numerous cross-sectional studies. The three available prospective studies support an association between maternal blood Pb and smaller head circumference from 1 to 4 years of age (Rothenberg *et al.* 1993, Rothenberg *et al.* 1999, Schell *et al.* 2009). The three available prospective studies addressing the potential association between maternal blood Pb and height or weight do not support an association (Lamb *et al.* 2008, Schell *et al.* 2009), although maternal Pb may be a contributing factor along with infant blood Pb levels (Shukla *et al.* 1989). The prospective studies that investigated the relationship between early-life blood Pb levels in children and measures of growth support a relationship between higher Pb and lower growth. The two Rothenberg *et al.* (1993, 1999) publications from the Mexico City Prospective Study reported an association between higher blood Pb in infants at 1 year of age and smaller head circumference up to 4 years of age, and Greene *et al.* (1991) reported that blood Pb in 1-year-old children was related to subsequent head circumference ($p=0.05$), length ($p=0.06$), and weight ($p=0.08$) in children in the Cleveland Lead Study. The strength of the evidence comes from the number of cross-sectional studies, including large numbers of subjects, such as the NHANES data sets, that report an inverse association between concurrent blood Pb (mean, 2-15 µg/dL) in children from 1 to 16 years of age and height or other indicators of postnatal growth. However, cross-sectional studies have considerable limitations because they only provide concurrent blood Pb measurements and lack data on cumulative Pb or Pb exposure at earlier time points that may be critical for growth. The consistency of effects across studies, among multiple populations in both males and females, lends weight to the evidence that decreased growth indicated by reduced height (and, to a lesser extent, other measures) is associated with blood Pb. The conclusion of *sufficient* evidence that blood Pb levels <10 µg/dL in children are associated with decreased growth is based on the combination of strong support from the cross-sectional studies and the additional support from the four prospective studies that evaluate blood Pb levels in children on subsequent growth. Although several studies report an association down to blood Pb levels <5 µg/dL, these studies do not adequately control for parental height, which is a known important predictor for postnatal growth, so there is inadequate data to evaluate an association with blood Pb levels <5 µg/dL. Data from the Cincinnati Lead Study suggest that

growth may depend on a combination of gestational and early-childhood exposure, so effects may only be observed in children of mothers with elevated blood Pb that subsequently experienced elevated blood Pb levels in early childhood. The conclusion of *limited* evidence that maternal blood Pb <10 µg/dL is associated with decreased head circumference in children up to 4 years of age is based on three studies from two groups. The NTP's conclusions on an inverse association between blood Pb and growth are in line with the 2007 ATSDR Toxicological Profile for Lead, although ATSDR does not specifically identify an effect level for growth, and the EPA's 2006 AQCD for Lead (U.S. EPA 2006) reviews the animal data in greater detail than the epidemiological data relating to growth.

8.3.3 Sperm

There is *sufficient* evidence that blood Pb ≥15 µg/dL is associated with adverse effects on sperm or semen in adult men, and *inadequate* evidence for adverse effects on sperm at blood Pb levels <15 µg/dL. Although there is no single measure of adverse effects that is consistently associated with elevated blood Pb, occupational studies report effects that include lower sperm numbers, decreased motility, reduced semen volume, and an increased percentage with abnormal morphology. Decreased sperm count, density, and/or concentration have been reported in multiple retrospective and cross-sectional studies of men with occupational exposure to Pb at mean blood Pb levels from 15 to 68 µg/dL (**Table 8.5** and Sperm section of Appendix E: Reproductive and Developmental Effects). Among men recruited from infertility or in vitro fertilization (IVF) clinics, decreased sperm concentrations and increases in the percentage of abnormal sperm are associated with blood Pb levels from 1 to 15 µg/dL in several studies (Chia *et al.* 1992, Telisman *et al.* 2007, Meeker *et al.* 2008). Men recruited from infertility clinics may represent a susceptible subpopulation, and even within this group the evidence is not consistent, because several studies did not find adverse sperm effects at blood Pb levels <15 µg/dL (mean, 7-10 µg/dL) (Xu *et al.* 1993, Mendiola *et al.* 2011). The conclusion of *inadequate* evidence that blood Pb levels <15 µg/dL are associated with adverse effects on sperm is based on the limited number of studies with evidence of effects at these lower blood Pb levels, the lack of consistency in the sperm data from men attending infertility or IVF clinics, and the uncertainty in using this patient group to extrapolate to other groups. There are few studies of the relationship between sperm and blood Pb in the general population.

Twelve occupational studies report adverse effects on sperm at blood Pb levels of 15-50 µg/dL using mean blood Pb levels of occupationally exposed men or blood Pb levels of workers categorized by blood Pb levels. Lower sperm counts or concentration are associated with the following blood Pb concentrations: approximately 15 µg/dL (estimated from graphs presented) in Naha *et al.* (2005); 20 µg/dL in De Rosa *et al.* (2003); approximately 25 µg/dL (estimated from graphs presented) in Telisman *et al.* (2000); 31 µg/dL in Mahmoud *et al.* (2005); ≥40 µg/dL in Alexander *et al.* (1996b); ≥44 µg/dL in Bonde *et al.* (2002); 48 µg/dL in Naha *et al.* (2006); and 50 µg/dL in Naha *et al.* (2007). Sperm motility is reduced at blood Pb levels of 20 µg/dL in De Rosa *et al.* (2003); 41 µg/dL blood Pb in Lancranjan *et al.* (1975); 49 µg/dL blood Pb in Lerda (1992); 53 µg/dL blood Pb in Kasperczyk *et al.* (2008); and 15 µg/dL, 48 µg/dL, and 50 µg/dL in

Table 8.5: Studies of sperm and semen parameters associated with low-level Pb exposure used to develop conclusions				
Relevance to Conclusions	**Study Description**	**Study Design**	**Key Reproductive/Developmental Findings**	**Reference**
Smelter employees, British Columbia		Retrospective	Blood Pb levels (>40 µg/dL) were associated with decreased sperm concentration, *but not with motility or morphology.*	Alexander (1996b)
Effect	Male employees; n=119			
	Male employees; n=81 of 119 original	Retrospective	Blood Pb levels (mean, 22.8 µg/dL) were associated with decreased sperm count and concentration, but *not with motility or morphology.*	Alexander (1998)
Effect	Male Pb workers in Europe; n=486	Cross-sectional	Blood Pb (>50 µg/dL) was associated with lower sperm count and density, *but not with volume, density ≤20 million/mL, or chromatin* in Pb workers (n=306; mean blood Pb, 31 µg/dL) and 197 referents (mean blood Pb, 4.4). The authors suggest a threshold of 44 µg/dL blood Pb for association with sperm concentration.	Bonde (2002)
Effect	Men at clinic in Croatia; n=240	Cross-sectional	Blood Pb (median, 4.9 µg/dL) was associated with increased percentages of pathological sperm, wide sperm, round sperm, *but not with sperm count, density, viability, motility, or other measures,* in men at fertility clinic or donors for artificial insemination.	Telisman (2007)
No effect	Men at infertility clinic in China; n=221	Cross-sectional	Blood Pb (mean, 8 µg/dL) and semen plasma Pb (mean, 1.27 µg/dL) were *not correlated with sperm density, motility, viability, morphology, or semen volume* in men screened for infertility.	Xu (1993)
Effect	Men at fertility clinic in Michigan; n=219	Cross-sectional	Blood Pb (median, 1.5 µg/dL) was associated with a greater odds ratio for below-reference sperm concentration *but not with count, volume, motility, or morphology*, for men at an infertility clinic.	Meeker (2008)
Effect	Male Pb workers and referents; n= 200	Cross-sectional	Men with Pb exposure (blood Pb ≥41 µg/dL; n=100) showed reduced sperm motility and semen volume and increased abnormal morphology compared to technicians (n=50) and referents (n=50).	Lancranjan (1975)
No effect	Men at fertility clinic in Germany; n=190	Case-control	Blood Pb not reported; grouped by sperm concentration, motility, and percent normal morphology, there were no differences in semen Pb among 172 infertile men and 18 referents.	Jockenhovel (1990)
No effect	Men at fertility clinic in Finland; n=188	Cross-sectional	Blood Pb not reported; *sperm density, motility, and morphology did not differ* by semen Pb above and below 0.2 µg/dL.	Saaranen (1987)
Effect	Male smelter workers in Belgium; n=159	Cross-sectional	Sperm concentration was significantly reduced in Pb workers (mean blood Pb, 31 µg/dL; n=68) compared to hospital staff (referents; median, 3.4 µg/dL; n=91).	Mahmoud (2005)
Effect	Male Pb workers in Croatia; n=146	Cross-sectional	Blood Pb levels were associated with decreased sperm count (blood Pb ≥~25 µg/dL), decreased sperm density, increased abnormal head morphology, and other parameters in Pb workers (mean blood Pb, 39 µg/dL; n=98) and referents (mean, 11 µg/dL; n=51).	Telisman (2000)

Table 8.5: Studies of sperm and semen parameters associated with low-level Pb exposure used to develop conclusions				
Relevance to Conclusions	**Study Description**	**Study Design**	**Key Reproductive/Developmental Findings**	**Reference**
Effect	Male Pb workers in India; n=130	Cross-sectional	Occupational exposure (mean blood Pb, 48 µg/dL (n=30) and 77 µg/dL (n=50)) was associated with decreased sperm count, density, motility, semen volume, increased abnormal morphology, and other sperm changes compared to referents (mean blood Pb, 14 µg/dL; n=50)	Naha (2006)
Effect	Male battery workers in India; n=120	Cross-sectional	Occupational Pb exposure (blood Pb ≥~14 µg/dL; n=80) was associated with decreased sperm count, density, motility, semen volume, increased abnormal morphology, and other sperm changes compared to referents (blood Pb 7 µg/dL; n=40).	Naha (2005)
Effect	Male Pb workers in Italy; n=120	Cross-sectional	Blood Pb levels were associated with decreased sperm count in 39 employees of a Pb battery plant (mean Pb, 61 µg/dL) and 81 workers at a cement plant (mean, 18 µg/dL).	Assennato (1986, 1987)
Effect	Male paint workers in India; n=100	Cross-sectional	Occupational Pb exposure (mean blood Pb, ≥50 µg/dL; n=50) was associated with decreased sperm count, motility, semen volume, and increased abnormal morphology and DNA hyploidy, compared to non-occupationally exposed workers (n=50).	Naha (2007)
No effect	Male battery workers in England; n=97	Cross-sectional	*Sperm count, density, and motility were not associated with blood* (mean Pb, 53 µg/dL) *or semen Pb* (mean Pb, 9.6 µg/dL). Percent of normal sperm was reduced at p=0.06.	Robins (1997)
Effect	Men in IVF clinic in New York; n=96	Cross-sectional	Blood Pb not reported; semen plasma Pb (mean, 40 µg/dL) was associated with decrease in sperm motility, concentration, morphology, other sperm measures, and decreased IVF fertilization.	Benoff (2003a)
Effect	Male battery workers in Taiwan; n=80	Cross-sectional	Blood Pb levels (mean, 40 µg/dL) were associated with increased percent abnormal sperm and sperm head morphology, and DNA denaturation, *but not with sperm count, semen volume, or motility*.	Hsu (2009)
Effect	Male battery workers in Argentina; n=68	Cross-sectional	Male Pb battery workers with blood Pb ≥49 µg/dL (n=38) show reduced sperm motility and semen volume and increased abnormal morphology compared to referents (n=30).	Lerda (1992)
Blood Pb: No effect; Semen Pb: Effect	Men living near a smelter in Mexico; n=68	Cross-sectional	Blood Pb (mean, 9 µg/dL) was *not associated with sperm parameters*. Sperm Pb (0.05 ng/10^6 cells) was associated with decreased sperm concentration, morphology, viability, motility; semen Pb (mean, 0.2 µg/dL) was associated with decreased volume and increased nuclear chromatin condensation.	Hernandez-Ochoa (2005)
Blood Pb: No effect; Semen Pb: Effect	Men at fertility clinic in Spain; n=60	Cross-sectional	*Sperm motility, concentration, and morphology did not differ by blood Pb* (mean, 9.8 µg/dL) for men at infertility clinic (n=30) and referents (n=30); motility was inversely related to semen Pb (3.0 µg/dL).	Mendiola (2011)
Effect	Male metal workers in Poland; n=63	Cross-sectional	Percent motile sperm was decreased in workers with high blood Pb (>40 µg/dL; n=29; mean Pb, 53 µg/dL) compared to workers with low Pb (<40 µg/dL; n=20) or referents (mean, 8 µg/dL; n=14); *semen volume, sperm count, and morphology did not differ*.	Kasperczyk (2008)

Table 8.5: Studies of sperm and semen parameters associated with low-level Pb exposure used to develop conclusions				
Relevance to Conclusions	**Study Description**	**Study Design**	**Key Reproductive/Developmental Findings**	**Reference**
No effect	Men in China; n=56	Cross-sectional	Blood Pb not reported; semen plasma Pb (mean, 0.78 µg/dL) was not correlated with sperm count, density, motility, morphology, or viability; semen Pb was associated with 8-OHdG in men (n=56; group characteristics not reported).	Xu (2003)
Equivocal	Men at infertility clinic in Europe; n=47	Cross-sectional	Blood Pb not reported; authors state inverse correlation between Pb and flagellum ball, and that no correlation was detected between pathological changes and elements.	Slivkova (2009)
Effect	Men in andrology clinic in China; n=35	Cross-sectional	Blood Pb was elevated (mean, 7.2 µg/dL) in men with <40% sperm motility compared to men with >40% sperm motility (mean blood Pb, 5.1 µg/dL) .	Chia (1992)
No effect	Men in Germany; n=22	Cross-sectional	Blood Pb not reported; Pb in semen (0.98 µg/dL) and semen plasma (0.77 µg/dL) *was not correlated to sperm density, count, motility, or morphology in men with no occupational Pb exposure*.	Noack-Fuller (1993)
No effect	Men in Connecticut; n=21	Cross-sectional	*No correlation between semen Pb* (mean, 5.9 µg/dL) *and sperm count, density, or semen protein in medical students and technicians*; unclear if blood Pb (13.1 µg/dL) effect on sperm examined.	Plechaty (1977)
Effect	Male Pb workers in India; n=20	Cross-sectional	Men with occupational Pb exposure (average blood Pb, 42.5 µg/dL; n=10) had lower sperm count, decreased percent motile sperm, and increased percent abnormal sperm compared to referents (n=10).	Chowdhury (1986)
Effect	Male battery workers in Netherlands; n=19	Cross-sectional	Decrease in blood Pb (median, 42-19 µg/dL) was associated with improved number of motile sperm and penetration in men undergoing treatment to lower blood Pb.	Viskum (1999)
Equivocal	Men with high Pb and referents; n=19	Cross-sectional	Men with chronic high occupational Pb exposure (mean blood Pb, 39 µg/dL; n=10) did *not differ from referents* (Pb=16 µg/dL; n=9) *in sperm volume, motility, or percent abnormal*; two men with highest Pb demonstrated peritubular fibrosis, oligospermia, and Sertoli cell vacuolization.	Braunstein (1978)
Effect	Male semen donors in New York; n=15	Cross-sectional	Blood Pb not reported; semen plasma Pb was associated with sperm motility, premature acrosome loss, and fertilization rate in IVF but not with sperm concentration.	Benoff (2003b)
Effect	Men with Pb toxicity in Connecticut; n=7	Case-series	Two of 7 men with occupational Pb intoxication had no sperm; 2 had reduced sperm count; 4 of the 5 with sperm had reduced sperm motility.	Cullen (1984)
Effect	A firearms instructor in New York	Case Report	A case report of increases in sperm density and total sperm count and decreases in abnormal morphology in parallel with decreasing blood Pb with chelation therapy in a 41-year-old.	Fisher-Fischbein (1987)

Epidemiological studies of Pb exposure and sperm effects listed by decreasing cohort size and grouped together for overlapping or shared study groups
Abbreviations: 8-OHdG, 8-hydroxydeoxyguanosine; IVF, in vitro fertilization.

three studies already listed above for decreased sperm count (Naha *et al.* 2005, Naha and Chowdhury 2006, Naha and Manna 2007). Five of the studies support effects in men with mean blood Pb levels from 15 to 31 µg/dL. Hsu *et al.* (2009) reported a threshold for increased abnormal sperm morphology at blood Pb levels ≥45 µg/dL in Pb battery workers, relative to workers with blood Pb <25 µg/dL. However, the extent of DNA denaturation per cell was significantly increased at lower blood Pb levels, among workers in both the mid-Pb group (25-45 µg/dL) and high-Pb group (>45 µg/dL) workers (Hsu *et al.* 2009). A similar threshold of approximately 25µg/dL was associated with decreased sperm count relative to workers with blood Pb <10 µg/dL in a study of 146 industrial workers in which Pb was dichotomized into six groups (n=25 per group) with mean blood Pb levels of <10, 12, 25, 35, 42, and 58 µg/dL (estimated from Figure 4 in Telisman *et al.* (2000)). Naha *et al.* (2005) reported effects on sperm in Pb workers with mean blood Pb of approximately 15 µg/dL (estimated from data presented in a graph; n=10 per group) compared to workers with mean blood Pb of 7 µg/dL. The Pb workers in the Naha *et al.* (2005) study had the lowest mean blood Pb level (15 µg/dL compared to 48-50 µg/dL) in a series of papers from the same group (Naha *et al.* 2005, Naha and Chowdhury 2006, Naha and Manna 2007) that consistently report effects on sperm count, motility, semen volume, and abnormal morphology in all Pb-exposed groups working in paint and battery factories compared to a reference group not working in the factory. A cross-sectional study of 68 Pb smelter workers reported reduced sperm concentration in the Pb workers (mean blood Pb, 31 µg/dL) compared to 91 hospital personnel (mean blood Pb, 3.4 µg/dL) (Mahmoud *et al.* 2005). De Rosa *et al.* (2003) reported lower sperm motility, viability, penetration, and velocity in 85 tollgate workers with mean blood Pb of 20 µg/dL compared to referents with mean blood Pb of 7 µg/dL, and linear regression for sperm count was also significant. The occupational studies of Naha *et al.* (2005), Telisman *et al.* (2000), Mahmoud *et al.* (2005), De Rosa *et al.* (2003) and Hsu *et al.* (2009) report adverse sperm effects down to blood Pb levels of 15-31 µg/dL.

A closer examination of the reference groups in the above studies adds to evidence for a threshold closer to 20 µg/dL for adverse effects on sperm than a threshold of 40 µg/dL; however, the data are not consistent. Of the seven studies that report the higher threshold, five rely on internal reference groups with blood Pb levels >10 µg/dL (Lerda 1992, Alexander *et al.* 1996b, Naha and Chowdhury 2006, Naha and Manna 2007) or fail to report blood Pb levels in the reference group (Lancranjan *et al.* 1975), so these studies have limited ability to detect effects in the lower concentration range. Among the six studies with a reference group <10 µg/dL, four reported effects on sperm at mean blood Pb levels from 15 to 31 µg/dL: ~15 µg/dL in Naha *et al.* (2005), 20 µg/dL in De Rosa *et al.* (2003), ~25 µg/dL in Telisman *et al.* (2000), and 31 µg/dL in Mahmoud *et al.* (2005). The other two studies (Bonde *et al.* 2002, Kasperczyk *et al.* 2008) report effects in men with blood Pb levels >40 µg/dL and not in men with blood Pb <40 µg/dL. While adjustment for potential confounders is not described in several of the studies, the studies of Telisman *et al.* (2000), Mahmoud *et al.* (2005), and Bonde (2002) are all adjusted for factors known to effect sperm count or function, such as age and period of abstinence, so a lack of adjustment does not explain the difference in the effect levels identified in the studies.

In addition to the occupational Pb exposure studies, there are a number of studies of individuals attending infertility or IVF clinics. Three of these studies reported an association between blood Pb levels <10 µg/dL and several sperm parameters, whereas two studies with similar blood Pb levels did not. In a cross-sectional study of 240 Croatian men that combined men at an infertility clinic with men donating for artificial insemination, blood Pb in the range of 1.1-14.9 µg/dL was associated with increasing percentage of pathologic sperm and wide sperm, with no effect on motility, viability, count, or other measures (Telisman *et al.* 2007). In a study of 219 men attending infertility clinics in Michigan, blood Pb was marginally (p-trend=0.07) related to sperm concentration below reference (<20 mil./mL) (Meeker *et al.* 2008). The odds ratio for low sperm concentration compared to the reference was significant for individuals in the second and third quartile compared to the first quartile: (second quartile, 1.1-1.5 µg/dL: OR=0.89 (95% CI: 0.27, 2.89); third quartile, 1.5-2.0 µg/dL: OR=3.94 (95% CI: 1.15, 13.6); fourth quartile, >2.0 µg/dL: OR=2.48 (95% CI: 0.59, 10.4) (Meeker *et al.* 2008). Chia *et al.* (1992) reported significantly elevated blood Pb (mean, 7.2 (SD: 6.2) µg/dL vs. 5.1 (SD: 2.4) µg/dL; p=0.0034) in men with <40% sperm motility among 35 men attending an andrology clinic in Singapore. The other two studies that report blood Pb and sperm parameters for men attending infertility clinics in China (n=221 at mean blood Pb of 8 µg/dL) and Spain (n=60 at mean blood Pb of 10 µg/dL) did not find an association between blood Pb and effects on sperm (Xu *et al.* 1993, Mendiola *et al.* 2011); however, Mendiola *et al.* (2011) reported an association between semen Pb levels and increased percentage of immotile sperm.

There are few studies of sperm effects associated with blood Pb levels in the general population that were not patients at infertility clinics, and the available studies do not support an effect of blood Pb on sperm. Hernandez-Ochoa *et al.* (2005) and Plechaty *et al.* (1977) did not detect a significant association between blood Pb (means, 9 and 13 µg/dL, respectively) and sperm parameters, but the studies are relatively small, with fewer than 89 men sampled from the two studies combined. It is also worth noting that two Pb-treatment studies support the inverse association between high blood Pb levels and sperm parameters. Motility, penetration, and morphology were all improved in 19 Danish Pb-workers treated for high Pb levels in association with lowering blood Pb from a median of 42 µg/dL to 19.9 µg/dL (Viskum *et al.* 1999). Similar results were observed in a case-report of a firearms instructor with blood Pb levels that were reduced from 88 to 30 µg/dL (Fisher-Fischbein *et al.* 1987).

In studies that report semen Pb levels, the results are inconsistent on whether semen Pb levels are associated with sperm parameters or whether semen Pb is a better measure of exposure than blood Pb for effects of Pb on sperm. Five reported sperm effects associated with semen Pb (Benoff *et al.* 2003a, Benoff *et al.* 2003b, Hernandez-Ochoa *et al.* 2005, Slivkova *et al.* 2009, Mendiola *et al.* 2011), and six did not find an association (Plechaty *et al.* 1977, Saaranen *et al.* 1987, Jockenhovel *et al.* 1990, Noack-Fuller *et al.* 1993, Robins *et al.* 1997, Xu *et al.* 2003). Several studies (e.g., Saaranen *et al.* 1987, Jockenhovel *et al.* 1990, Noack-Fuller *et al.* 1993, Benoff *et al.* 2003a, Benoff *et al.* 2003b, Xu *et al.* 2003, Slivkova *et al.* 2009) report only semen or sperm Pb, so the usefulness of semen Pb cannot be compared to blood or bone Pb as a potential biomarker of Pb exposure related to sperm parameters. In studies that report both blood and semen Pb, there is some support that both measures of exposure are equally good

indicators of Pb exposure, because as several studies report sperm effects associated with both blood and semen Pb (Telisman *et al.* 2000, Naha *et al.* 2005, Naha and Chowdhury 2006, Naha and Manna 2007) or a lack of an association that is consistent with both blood and semen Pb (Xu *et al.* 1993, Robins *et al.* 1997). Other studies have reported a significant association of sperm parameters with semen Pb (and not blood Pb) (Hernandez-Ochoa *et al.* 2005, Mendiola *et al.* 2011) or blood Pb (and not semen Pb) (Assennato *et al.* 1986, Kasperczyk *et al.* 2008). In a study of 81 employees of the Cominco smelter in British Columbia, semen and blood Pb levels were associated with decreased sperm concentration; however, after adjustment for ejaculate volume, blood Pb remained significant and semen Pb levels were no longer significantly related to sperm concentration (Alexander *et al.* 1998).

Summary of support for conclusions

Animal data support adverse effects of Pb exposure on sperm and semen, including decreased sperm count, reduced sperm motility, and increased morphological abnormalities, in sperm in some studies (see U.S. EPA 2006 for recent reviews of the animal data, ATSDR 2007). Although the animal data generally support adverse effects of Pb on sperm, effects may be associated with high doses (40-75 µg/dL in rats), and the animal data display differences in sensitivity to sperm effects by species and strain (effects observed down to 16-24 µg/dL in rabbits), differences that are likely to be exacerbated by variation in route and duration of exposure, age at initial exposure, and chemical form of Pb used in the experiment. The human data include 12 studies of men with occupational exposure that report effects on sperm or semen at blood Pb levels from 15 to 50 µg/dL. This is supported by six studies reporting effects on time to pregnancy or fertility at similar Pb levels (10-46 µg/dL) in men described in **Section 8.3.4 Fertility / Delayed Conception Time** below. The conclusion of *sufficient* evidence that blood Pb levels ≥15 µg/dL are associated with adverse effects on sperm or semen is based on these studies and specifically the five studies that report effects on sperm at blood Pb levels from 15 to 31 µg/dL. Although occupational studies support adverse sperm effects down to 15 µg/dL, the lower threshold of blood Pb level associated with these effects is unclear.

Several studies of men recruited from IVF or infertility clinics report effects at blood Pb levels <10 µg/dL. However, men recruited from infertility clinics may represent a susceptible subpopulation, and even within this group, the evidence is not consistent. One challenge in determining the lower limit is that much of the data come from occupational studies in which the mean blood Pb level is 30-40 µg/dL. Also, as discussed above, there are few studies of effects in the general population, and many studies have a low ability to detect effects associated with lower blood Pb levels, because occupational studies generally do not include many men with lower blood Pb levels (i.e., blood Pb <10 µg/dL). A number of older studies of sperm or semen parameters (e.g., Lancranjan *et al.* 1975, Lerda 1992) do not adjust for confounding factors such as period of abstinence, age, and smoking. In addition, no human data were located that examine effects of early or developmental exposure on sperm parameters as adults. The NTP's conclusion of *sufficient* evidence that blood Pb levels ≥15 µg/dL are associated with adverse effects on sperm or semen extends the conclusions of the 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) and 2006 EPA AQCD for Lead (U.S.

EPA 2006) down to 15 μg/dL blood Pb from the range of 30-45 μg/dL suggested by the 2006 EPA and 2007 ATSDR documents.

8.3.4 Fertility / Delayed Conception Time

There is *sufficient* evidence that paternal blood Pb levels ≥20 μg/dL are associated with delayed conception time and *limited* evidence that blood Pb levels ≥10 μg/dL in men are associated with other measures of reduced fertility. Four studies reported increased time to pregnancy or decreased odds of conception over a given time (fecundability) in men with blood Pb levels from 20 to 40 μg/dL, and a fifth study reported decreased odds ratio for probability of live birth in Pb workers with mean blood Pb of 46 μg/dL (see Fertility /Delayed Conception Time section of Appendix E: Reproductive and Developmental Effects). A lower effect level of 10 μg/dL is supported by a single large (n=4,146) retrospective occupational study that reported increased odds of infertility among men with blood Pb ≥10 μg/dL (Sallmen *et al.* 2000b), providing *limited* evidence for effects on fertility at blood Pb levels down to 10 μg/dL in men. The database of studies that examined male Pb exposure and fertility also includes several occupational studies that did not observe a significant relationship between blood Pb levels in men and fertility or time to pregnancy. There is *inadequate* evidence that blood Pb levels of ≤10 μg/dL in women are associated with decreased fertility or greater time to pregnancy because few studies address these effects, and all of them report that maternal blood Pb levels are not associated with time to pregnancy. There is one prospective study of time to pregnancy for women in the general population with blood Pb levels <10 μg/dL, and this study found no effect on time to pregnancy. Several studies of couples attending IVF or infertility clinics report an association between blood Pb levels from 1 to 30 μg/dL and a decrease in some measure of fertility (e.g., fertilization or embryo quality); additional studies are required to confirm this relationship. The best studies of fertility and delayed conception time include exposure measurements in both partners, but these data are often not collected. Women and men recruited from infertility or IVF clinics may represent a susceptible subpopulation, and the effects observed in this patient population should be applied with caution to the general population. There are not enough studies of fertility with Pb exposure data for women in the general population or even with occupational exposure to evaluate the potential relationship between Pb exposure and fertility in women.

Six occupational studies in five groups report decreased fertility or greater time to pregnancy for men at blood Pb levels of 10-46 μg/dL, and four other studies report no association at similar blood Pb levels. In a cross-sectional study of 85 tollgate workers with blood Pb levels of 20 μg/dL compared to 85 referents with blood Pb of 7 μg/dL, De Rosa *et al.* (2003) reported a significant increase from 8 to 15 months in time to pregnancy. Male workers (n=133) in a Pb battery plant in Taiwan had delayed time to pregnancy and reduced odds of conception over a given time compared to referents (fecundability ratio (FR)) at blood Pb levels ≥30 μg/dL (blood Pb 30-39 μg/dL: FR=0.52 (95% CI: 0.35, 0.77); blood Pb ≥40 μg/dL: FR=0.40 (95% CI: 0.27, 0.59); (Shiau *et al.* 2004). In retrospective occupational exposure studies of men monitored for Pb exposure by the Finnish Institute of Occupational Health, Sallmen *et al.* (2000a) reported the decreased FR relative to the reference group (FR=0.57 (95% CI: 0.34-0.91)) among men (n=502) with blood Pb ≥31 μg/dL in analyses that included only full-term pregnancies. Apostoli *et al.*

(2000) reported a significant delay in time to pregnancy for men with blood Pb ≥40 µg/dL (p=0.012) in a study of 251 men working at a Pb-related factory in Italy; however, the time to pregnancy was not reduced at lower blood Pb levels, and FR analysis suggests a shorter time to pregnancy at lower blood Pb levels. In a cross-sectional study of 365 male Pb battery plant workers with mean blood Pb of 46 µg/dL, the odds ratio for probability of live birth was decreased compared to workers with mean blood Pb of 10 µg/dL (OR=0.65 (95% CI: 0.43, 0.98)) or relative to preexposure (OR=0.43 (95% CI: 0.25, 0.73)) (Gennart *et al.* 1992b). The five studies described above support greater time to pregnancy or reduced fertility in men at blood Pb levels of 20 µg/dL, 30 µg/dL, 31 µg/dL, 40 µg/dL, and 46 µg/dL, and a large retrospective study reported increased odds ratio of infertility at even lower blood Pb levels, down to 10 µg/dL. In a retrospective studies of men monitored for Pb exposure by the Finnish Institute of Occupational Health, Sallmen *et al.* (2000b) reported increased odds of infertility (RR=1.27 (95% CI: 1.08, 1.51)) and decreased success ratio (SR=0.86 (95% CI: 0.77, 0.97) for pregnancy among wives of male workers (n=4,146) with blood Pb ≥10 µg/dL. Male Pb workers reporting to the New York State Heavy Metals Registry with more than 5 years of Pb work had a reduced fertility rate relative to bus drivers or Pb workers with <5 years of occupational exposure; however, blood Pb levels alone were not related to fertility (Lin *et al.* 1996). There are also four retrospective studies that report no association between time to pregnancy or odds ratio for fertility in men occupational exposed to Pb. Paternal Pb was not associated with standardized fertility ratio in 376 male Pb battery workers compared to preemployment group or workers with blood Pb <25 µg/dL (Selevan *et al.* 1984). Odds ratios for reduced fertility did not differ between 1,349 Danish Pb workers with a mean blood Pb of 36 µg/dL compared to 9,596 referents (without blood Pb data) (Bonde and Kolstad 1997). The odds ratios for infertility did not differ between 229 Pb workers categorized by blood Pb into three groups of <40, 40-60, and >60 µg/dL and 125 reference employees classified as nonexposed (Coste *et al.* 1991). Time to pregnancy was not increased in 638 Pb-exposed male workers (mean Pb, 29-37 µg/dL) compared to external referents (n=236) or an internal control group (n=230) (Joffe *et al.* 2003).

Few studies have investigated the potential relationship between Pb exposure and fertility or time to pregnancy in women. There is one prospective study of time to pregnancy for women in the general population, Bloom *et al.* (2011a), which reported that blood Pb levels (mean, 1.5 µg/dL) were not associated with time to pregnancy in a study of 80 women in New York. Sallman *et al.* (1995) did not detect a relationship between odds of conception and maternal occupational blood Pb levels in a retrospective study of 121 women in which exposure was estimated based on work descriptions and limited biological measurements.

Several fertility studies report measures of exposure other than blood Pb levels. Three case-control studies of infertile men and two studies of men undergoing IVF examined semen Pb levels and did not report blood Pb data, so the usefulness of semen Pb cannot be compared to blood or bone Pb as a potential biomarker of Pb exposure related to sperm parameters. Semen Pb was higher in infertile men in two of the studies (Saaranen *et al.* 1987, Jockenhovel *et al.* 1990), but not in a third study (Umeyama *et al.* 1986). Benoff *et al.* (2003a, 2003b) reported an inverse correlation between semen plasma Pb and IVF rate in two studies at IVF/ artificial

insemination clinics in studies that did not include blood Pb values. Of the two studies reporting follicular Pb levels, one reported an association with fertility and one did not. In a study of 619 women undergoing IVF in Saudi Arabia, follicular Pb levels were not related to fertilization or pregnancy outcome, although blood Pb was associated with decreased OR for fertilization (Al-Saleh *et al.* 2008a). In a small study (n=9 women) that did not report blood Pb, follicular Pb was significantly higher from IVF patients that did not get pregnant than from women that did get pregnant (Silberstein *et al.* 2006).

Several studies of couples attending IVF or infertility clinics report an association between blood Pb levels and a decrease in some measure of fertility (e.g., fertilization or embryo quality). Results from studies of men or women reporting to IVF or infertility clinics should be interpreted with caution because they may represent a sensitive subpopulation. In a study of couples undergoing IVF in California, increased blood Pb levels in the women (n=24; mean blood Pb, 0.83 µg/dL) were associated with a decreased OR for higher embryo cell numbers (a measure of embryo quality) (OR=0.25 (95% CI: 0.07, 0.86)), or a 75% reduction in embryo quality for each 1 µg/dL increase in maternal blood Pb (Bloom *et al.* 2010, Bloom *et al.* 2011b). Increased blood Pb levels in the men (n=15; mean blood Pb, 1.5 µg/dL) were also associated with a decreased OR for higher embryo cell numbers (OR=0.58 (95% CI: 0.37, 0.91)), or a 42% reduction in embryo quality for each 1 µg/dL increase in paternal blood Pb. There are only two case-control studies of infertile patients with blood Pb levels: one comparing infertile men to fertile controls and one comparing infertile women to fertile controls. Blood Pb (36.8 µg/dL (SD: 12) vs. 23.2 µg/dL (SD: 5.6)) and semen Pb were higher in infertile men at a fertility clinic than in controls in a case-control study that examined Pb and smoking (Kiziler *et al.* 2007). Self-reported Pb exposure did not differ between infertile men recruited from infertility clinics and fertile men from prenatal clinics (Gracia *et al.* 2005). In a case-control study of women recruited at an infertility clinic (n=64) and controls from a postpartum clinic (n=83) in Taiwan, blood Pb levels >2 µg/dL were associated with an increased OR for infertility (OR=2.94 (95% CI: 1.18, 7.34)) (Chang *et al.* 2006). In a study of 619 women undergoing IVF, mean blood Pb levels were significantly higher in women that failed to achieve fertilization (4.1 (SD: 3.7) µg/dL) than in women in which the IVF produced fertilized eggs (3.26 (SD: 2) µg/dL); however, blood Pb was not related to pregnancy outcome (Al-Saleh *et al.* 2008a).

Summary of support for conclusions

Animal data support adverse effects of Pb on fertility in several studies at high blood Pb concentrations (>60µg/dL; see U.S. EPA 2006 for recent reviews of the animal data, ATSDR 2007). Effects in male rodents exposed to Pb before mating include increased time to birth in mice and decreased pregnancy rate in mice or rats (Gandley *et al.* 1999, Pace *et al.* 2005). Female mice exposed to Pb during pregnancy exhibited smaller litter size in several studies (e.g., Pinon-Lataillade *et al.* 1995). The human data include six studies of men with occupational exposure that report increased time to pregnancy or reduced fertility at blood Pb levels from 10 to 46 µg/dL. The conclusion of *sufficient* evidence that blood Pb levels ≥20 µg/dL are associated with delayed conception time is based on four studies reporting increased time to pregnancy with blood Pb levels of 20-40 µg/dL in men. This is supported by numerous

studies reporting adverse effects on sperm at similar Pb levels (15-50 µg/dL) in men described earlier. Although some studies do not support an association between paternal blood Pb levels and time to pregnancy or fertility, the database (including the sperm data) provides *sufficient* evidence for delayed conception time at blood Pb levels ≥20 µg/dL in men. The conclusion of *limited* evidence that blood Pb levels ≥10 µg/dL in men are associated with decreased fertility is based on the above data with the addition of a single large retrospective occupational study that reported increased odds of infertility among men with blood Pb levels ≥10 µg/dL (Sallmen *et al.* 2000b). In the human data, few studies examined fertility or time to pregnancy with Pb exposure data in women, and both studies that examined time to pregnancy reported that maternal blood Pb was not related to time to pregnancy. The conclusion of *inadequate* evidence that blood ≤Pb 10 µg/dL in women are associated with increased time to pregnancy or reduced fertility is based on the limited number of studies addressing these endpoints, and the lack of a significant association with blood Pb reported in some studies on time to pregnancy. The conclusion of *inadequate* evidence for effects on fertility in women is consistent with the 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) and EPA's 2006 AQCD for Lead; however, for men, the 2006 EPA AQCD for Lead (U.S. EPA 2006) states that epidemiological studies suggest a small association between blood Pb levels >45 µg/dL in men and increased time to pregnancy. The NTP conclusions of *sufficient* evidence for effects of blood Pb levels ≥20 µg/dL in men on time to pregnancy and *limited* evidence that blood Pb levels ≥10 µg/dL are associated with reduced fertility are consistent with the conclusions of an effect of Pb in the 2006 EPA and 2007 ATSDR Lead documents, but the NTP outlines the support for a lower effect level (i.e., 20 µg/dL rather than 30-45 µg/dL). While adjustment for potential confounders is not described in several of the studies, the studies of Shiau *et al.* (2004), Sallmen *et al.* (2000a), and Apostoli (2000) adjusted for factors known to effect fertility, such as maternal age and previous abortion, and all three studies demonstrated an effect of paternal Pb on time to pregnancy. Therefore, a lack of consideration of confounders does not explain the difference between studies that identified an effect of paternal Pb and studies such as Joffe *et al.* (2003) that did not observe an association between blood Pb in men and time to pregnancy.

8.3.5 Spontaneous Abortion

There is *limited* evidence that maternal blood Pb <10 µg/dL is associated with spontaneous abortion. In an extensive review, Hertz-Picciotto *et al.* (2000) concluded that there is consistent evidence from case series and epidemiologic studies in the 19th and early 20th century that Pb exposure at high levels appears to play a role in spontaneous abortion, but the older studies cited lack blood Pb or other biological monitoring data, so it is unclear what blood Pb level the data would support. Although >20 studies published since the 1970s address maternal or paternal Pb exposure and spontaneous abortion (see Spontaneous Abortion section of Appendix E: Reproductive and Developmental Effects), many lack biological monitoring data. Four of the five retrospective studies that determine exposure by employment or residence report an association between maternal Pb exposure and spontaneous abortion (Nordstrom *et al.* 1978, 1979, Driscoll 1998, Tang and Zhu 2003). Of the studies with blood Pb data, two studies report an association between maternal Pb levels and spontaneous abortion. One case-control study and one nested case-control study found an effect of maternal blood Pb or plasma Pb <10 µg/dL and increased risk of spontaneous abortion (Borja-Aburto *et al.* 1999, Yin

et al. 2008). A number of studies have reported no association between maternal blood Pb levels above or below 10 µg/dL and spontaneous abortion. The conclusion of *limited* evidence that maternal blood Pb <10 µg/dL is associated with spontaneous abortion is based principally on the Borja-Aburto *et al.* (1999) study, which has the strength of the prospective nested case-control design, and additional supporting evidence provided by the Yin *et al.* (2008) data, as well as the occupational studies without blood Pb measurements. There is *limited* evidence that paternal blood Pb >31 µg/dL is associated with spontaneous abortion. A positive association between paternal blood Pb >31 µg/dL was reported in one occupational study (Lindbohm *et al.* 1991a, Lindbohm *et al.* 1991b) and two retrospective studies that determined exposure by employment but lack blood Pb data (Beckman and Nordstrom 1982, Al-Hakkak *et al.* 1986). Two other studies have reported no association at similar blood Pb levels (i.e., >25µg/dL Selevan *et al.* 1984, Alexander *et al.* 1996a)). The conclusion of *limited* evidence that paternal blood Pb >31 is associated with spontaneous abortion is based mainly on the retrospective nested case-control study by Lindbohm *et al.* (1991a, 1991b), with support from the occupational studies without blood Pb measurements.

The principal evidence supporting an association between maternal blood Pb levels and spontaneous abortion relies primarily on the Borja-Aburto *et al.* (1999) prospective nested case-control study of women in Mexico. The study reported evidence for a dose response (p-trend=0.03) and significant ORs for spontaneous abortion (ORs of 2.3, 5.4, and 12.2) with maternal blood Pb during the first trimester of pregnancy of 5-9, 10-14 and ≥15 µg/dL compared to <5.0 µg/dL in the reference group (Borja-Aburto *et al.* 1999). The analysis highlighted the careful matching of the timing of exposure measurements in the 35 cases with controls and was adjusted for a range of potential confounders, including age, smoking, alcohol consumption, and physical activity. Four retrospective studies support an association between maternal Pb exposure and spontaneous abortion; however, no blood Pb data were included, and exposure was determined by employment or residence (Nordstrom *et al.* 1978, 1979, Driscoll 1998, Tang and Zhu 2003). Additional support is provided by a case-control study that reported plasma Pb levels with no blood Pb data; maternal plasma Pb (5.3 µg/dL in the 40 case women compared to 4.5 µg/dL in the 40 controls) was significantly higher in women with anembryonic pregnancy (i.e., a pregnancy that appears normal in early stages, with the embryo that is visible by ultrasound never developing (Yin *et al.* 2008)). However, the study reports high levels of plasma Pb suggesting either very high blood Pb levels or a potential problem in study performance or reporting. Several studies with mean blood Pb levels from 4 to 16 µg/dL reported no association between maternal blood Pb levels and spontaneous abortion. Maternal blood Pb during the first trimester was not associated with spontaneous abortion at mean blood Pb levels of 4 µg/dL in a recent prospective study of 351 women in Iran (Vigeh *et al.* 2010). A prospective study of women residing in a Pb-smelting community reported that maternal blood Pb was not statistically different between cases of spontaneous abortion (11.3 µg/dL) and controls (10.8 µg/dL) (McMichael *et al.* 1986). Two retrospective studies that compared recalled pregnancy outcomes and concurrent blood Pb levels (means of 6 µg/dL in Lamadrid-Figueroa *et al.* (2007) and 16 µg/dL in Murphy *et al.* (1990)) reported that concurrent blood Pb in women was not associated with previous history of spontaneous abortion.

The principal evidence supporting an association between paternal blood Pb levels and spontaneous abortion relies on three studies that report an association with paternal Pb exposure. In a retrospective analysis of blood Pb measurements in men occupationally exposed to Pb restricted to within 1 year of the spermatogenesis period relevant to a given pregnancy, paternal blood Pb >31 µg/dL was associated with higher odds ratio of spontaneous abortion (OR=3.8 (95% CI: 1.2, 12)) compared to men with blood Pb <21 µg/dL (Lindbohm *et al.* 1991b). Two retrospective studies of men with occupational exposure to Pb also reported an association between paternal Pb exposure and spontaneous abortion; however, exposure was determined by employment, and the studies lack blood Pb data (Beckman and Nordstrom 1982, Al-Hakkak *et al.* 1986). Two additional occupational studies that include blood Pb levels did not detect an association between spontaneous abortion and paternal blood Pb from 25 to >60 µg/dL (Selevan *et al.* 1984, Alexander *et al.* 1996a).

In analyses of other biomarkers of Pb exposure, Figueroa *et al.* (2007) found that women with higher ratio of Pb in plasma to Pb in blood had a greater incidence rate for previous abortion (incidence rate ratio=1.18; p=0.02 for 1 SD increase); however, when examined individually, neither blood, plasma, tibia, nor patella Pb levels were related to spontaneous abortions. In a case-control study that also reported plasma Pb levels, maternal plasma Pb (5.3 µg/dL in the 40 case women compared to 4.5 µg/dL in the 40 controls) was significantly higher in women with anembryonic pregnancy (Yin *et al.* 2008); however, the study does not report Pb levels in whole blood, reports high levels of plasma Pb suggesting either very high blood Pb levels or a potential problem in study performance or reporting, and ratios of Pb in plasma to Pb in whole blood vary widely (from 0.27% to 0.70%), so it is unclear how plasma Pb data relates to the blood Pb data described above (Hernandez-Avila *et al.* 1998). Placental Pb was significantly higher in women that had a previous miscarriage (Gundacker *et al.* 2010).

Summary of support for conclusions

Animal data were not located that support an association between Pb and spontaneous abortion, although prenatal exposure to Pb has been associated with decreased litter sizes, decreased pup survival, and increased embryonic resorption at very high blood Pb levels (>200µg/dL in mice and rats; see U.S. EPA 2006 for recent reviews of the animal data, ATSDR 2007). As described above, there are few human studies with blood Pb data that evaluate the potential association with spontaneous abortion. The conclusions that there is *limited* evidence that maternal blood Pb <10 µg/dL and paternal blood Pb >31 µg/dL are associated with spontaneous abortion are based primarily on two key studies: the Borja-Aburto *et al.* (1999) prospective nested case-control study and Lindbohm *et al.* (1991a) retrospective nested case-control study. Additional support for the association is provided by several studies that determine exposure by occupation or residence rather than by blood Pb data. In addition, some studies with blood Pb data did not find an association between maternal or paternal blood Pb levels and spontaneous abortion. The inconsistency of the results contributes to the determination of *limited* evidence. Although not all studies considered confounders, a lack of adjustment for confounders does not appear to explain the lack of consistency, because studies that both supportive for Pb effects on spontaneous abortion (e.g., Borja-Aburto *et al.* 1999) and studies that are not supportive for effects of Pb (e.g., data from Vigeh *et al.* 2010) included

adjustments for maternal age, smoking, and other factors. The 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) and 2006 EPA AQCD for Lead (U.S. EPA 2006) both highlight the lack of consistency of the data on spontaneous abortion; however, the Borja-Aburto *et al.* (1999) study is highlighted as a well-conducted prospective case-control study supporting a significant relationship between maternal blood Pb and spontaneous abortion.

8.3.6 Stillbirth

There is *inadequate* evidence to evaluate the potential association between blood Pb at any level and incidence of stillbirth. Few studies investigate the potential association between Pb exposure and stillbirth, and only a handful have blood Pb or other biological monitoring data (see Stillbirth section of Appendix E: Reproductive and Developmental Effects). Of the studies with blood Pb data, none of the studies support an association between maternal or paternal blood Pb and stillbirth. For example, a prospective study of women residing in a Pb-smelting community reported that maternal blood Pb levels were not significantly different between cases of stillbirth (10.3 µg/dL during pregnancy and 7.2 µg/dL at delivery) and controls (9.9 µg/dL during pregnancy and 10.4 µg/dL at delivery) (McMichael *et al.* 1986). In a retrospective study that compared recalled pregnancy outcome and concurrent blood Pb levels (means of 16 µg/dL in the residents in a Pb-smelter community and 5.1 µg/dL in referents), Murphy *et al.* (1990) reported that concurrent blood Pb was not associated with previous history of stillbirth (OR=1.0 (95% CI: 0.6, 1.5)). There are some examples of Pb-associated increases in stillbirth in animal literature at very high doses (e.g., >200µg/dL in Sprague-Dawley rats; see U.S. EPA 2006 for recent reviews of the animal data, ATSDR 2007). The data set available to evaluate this association is small and includes a single prospective study. The 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) and 2006 EPA AQCD for Lead (U.S. EPA 2006) do not have specific conclusions on the potential association between Pb exposure and stillbirth.

8.3.7 Fetal Growth and Lower Birth Weight

There are several measures of reduced prenatal growth or intrauterine growth restriction: small for gestational age (babies with birth weight below the 10th percentile for a given gestational age), lower birth weight (evaluated as a continuous variable), low birth weight (<2,500 g after at least 37 weeks of gestation), and low birth weight adjusted for gestation length. For this evaluation, any indication of reduced fetal growth is included below.

There is *sufficient* evidence that maternal blood Pb levels <5 µg/dL are associated with reduced fetal growth and lower birth weight. The association between maternal Pb exposure and reduced fetal growth is supported by a number of prospective studies with maternal blood Pb data during pregnancy (Dietrich *et al.* 1987, Bornschein *et al.* 1989, Jelliffe-Pawlowski *et al.* 2006, Gundacker *et al.* 2010), a large retrospective cohort study of over 43,000 mother-infant pairs with mean maternal blood Pb level of 2.1 µg/dL (Zhu *et al.* 2010), and a number of cross-sectional studies with maternal or umbilical cord blood Pb at delivery (Bellinger *et al.* 1991, Neuspiel *et al.* 1994, Odland *et al.* 1999, Osman *et al.* 2000, Srivastava *et al.* 2001, Chen *et al.* 2006, Zentner *et al.* 2006, Al-Saleh *et al.* 2008b) (see Fetal Growth and Lower Birth Weight

section of Appendix E: Reproductive and Developmental Effects). There is also one prospective study (Sowers *et al.* 2002) and several cross-sectional studies that report no association between maternal blood Pb and reduced fetal growth at blood Pb levels <10 µg/dL and similar results at higher blood Pb levels. Although the results are not entirely consistent across studies, the supporting evidence outlined above with maternal or umbilical cord blood Pb levels <10 µg/dL from multiple prospective, retrospective, and cross-sectional studies provides *sufficient* evidence at maternal blood Pb levels <10 µg/dL are associated with reduced fetal growth and lower birth weight. The large retrospective study of 43,288 mother-infant pairs from the New York State Heavy Metals Registry contributes substantially to the conclusions, because the study reported a significant association between maternal blood Pb (mean, 2.1 µg/dL) and lower birth weight in such a large cohort from a study that included adjustment for multiple potential confounders (Zhu *et al.* 2010). The evidence supporting an effect of maternal Pb exposure on reduced fetal growth is further strengthened by a number of studies from a population in Mexico demonstrating that maternal bone Pb is associated with lower birth weight, birth length, and head circumference. These studies provide limited evidence that maternal bone Pb levels >15.1 µg/g (tibia Pb) are associated with reduced fetal growth. Unfortunately, the evidence to evaluate the potential association between maternal bone Pb and low birth weight is restricted to a single group. All five of the studies that include maternal bone Pb measurements report a significant association between increased maternal bone Pb levels and lower fetal growth, but they are all studies of women attending one of three hospitals in Mexico City from 1994 to 1995. There is *inadequate* evidence that paternal blood Pb at any level is associated with reduced fetal growth, because few studies of birth weight or related endpoints have included paternal Pb exposure data, and the available studies do not support an association with blood Pb in men.

Most prospective or retrospective studies that evaluated the association between maternal blood Pb levels of ≤10 µg/dL during pregnancy with measures of fetal growth found an inverse association (i.e., higher Pb levels were related to reduced fetal growth), although one study did not find an effect of blood Pb. Maternal blood Pb (mean, 2.5 µg/dL) at 34-38 weeks of gestation (n=53) in women at General Hospital in Vienna was associated with lower birth weight (Gundacker *et al.* 2010). Maternal blood Pb levels (mean, 7.5 µg/dL) of 861 women from the Cincinnati Lead Study at 16-28 weeks of gestation (from the second trimester into the start of the third trimester) were associated with decreased birth weight (Bornschein *et al.* 1989). This result was also supported in a smaller analysis: higher maternal blood Pb levels (mean, 8.3 µg/dL) sampled at the first prenatal visit in women (n=185) from the Cincinnati Lead Study were correlated with lower birth weight (Dietrich *et al.* 1987). Maternal blood Pb ≥10 µg/dL during pregnancy in women in the California Pb surveillance program (n=262) was associated with a greater odds ratio for small for gestational age (OR=4.2 (95% CI: 1.3, 13.9)); however, the association was not significant when analyzed for low birth weight (OR=3.6 (95% CI: 0.3, 40)) (Jelliffe-Pawlowski *et al.* 2006). Maternal blood Pb (mean, 2.1 µg/dL) during pregnancy or at birth was associated with lower birth weight in a large retrospective study of 43,288 mother-infant pairs from the New York State Heavy Metals Registry (Zhu *et al.* 2010). The large retrospective study of over 43,000 mother-infant pairs from Zhu *et al.* (2010) contributes substantially to the conclusions because the study reported a significant association

between a maternal blood Pb level well below 10 µg/dL and lower birth weight in very large cohort from a study that included adjustment for multiple potential confounders, including maternal age, race, parity, smoking, drug abuse, infant sex, and participation in financial assistance as a measure of socioeconomic status (Zhu *et al.* 2010). Maternal blood Pb (mean, 1.1 µg/dL) sampled at 12, 20, and 28 weeks of gestation, and the change in blood Pb levels over pregnancy in 705 women in Camden, NJ, were not associated with low birth weight or small for gestational age (Sowers *et al.* 2002). Other than the clear difference in the sample size for the Zhu *et al.* study (2010), there is no obvious difference in blood Pb levels, or in statistical adjustments, between the five studies that support an effect of maternal blood Pb levels <10 µg/dL and the one that does not.

Additional studies that sampled maternal or umbilical cord blood Pb at delivery also reported an association between blood Pb levels of ≤10 µg/dL and measures of fetal growth. Studies of cohorts with higher mean blood Pb levels (i.e., >10 µg/dL) are not described below because the discussion focuses on the evidence at blood Pb levels <10 µg/dL. However, as with the prospective studies described above, the results are not consistent across all studies. The principal studies with maternal blood Pb levels at delivery are listed below. The data on umbilical cord blood and blood Pb and higher mean blood Pb levels (i.e., >10 µg/dL) are not detailed below because they present similar results, but all studies are included in the Fetal Growth and Lower Birth Weight section of Appendix E: Reproductive and Developmental Effects. Mean maternal blood Pb levels at delivery of 2-13 µg/dL were associated with lower birth weight in mother-infant pairs from a case-control study of 30 births with intrauterine growth restriction (referred to as intrauterine growth retardation by the authors) and 24 normal births in India (Srivastava *et al.* 2001); a combined population from Russia and Norway (n=262) (Odland *et al.* 1999); and a Pb surveillance program in Taiwan (n=72 low-birth-weight infants of 1,611 births) (Chen *et al.* 2006). Mean maternal blood Pb levels at delivery of 6-10 µg/dL were not associated with birth weight, birth length, or head circumference in mother-infant pairs from women in Cleveland (n=185) (Ernhart *et al.* 1986); Karachi (n=73) (Rahman and Hakeem 2003); or Mexico City (n=272-533) (Cantonwine *et al.* 2010b).

Few studies of fetal growth include paternal blood Pb levels, and the available evidence provides little support for an association with blood Pb in men. Paternal occupational exposure estimated by job category was associated with low birth weight and small for gestational age in a study of 742 births in the Baltimore-Washington Infant Study (Min *et al.* 1996). In two other studies that classified exposure by paternal job category, paternal occupation Pb exposure was not associated with low birth weight in a study of members of the printers' unions in Oslo, Norway (n=6,251 births) (Kristensen *et al.* 1993) or occupational exposure in Norway with possible paternal Pb exposure (n=35,930 births, although maternal Pb exposure was associated with low birth weight) (Irgens *et al.* 1998). Paternal blood Pb (mean, 13 µg/dL) was not associated with small for gestational age or low birth weight in data from a Pb surveillance program in Taiwan (n=72 low-birth-weight infants of 1,611 births) (Chen *et al.* 2006). Low birth weight was associated with maternal blood Pb levels in the Chen *et al.* (2006) study and occupational Pb exposure in the Irgens *et al.* (1998) study. Paternal blood Pb was not associated with birth weight or small for gestational age; however, blood Pb levels >25 µg/dL

for more than 5 years was associated with increased relative risk of low birth weight in a study of workers (n=747) reporting to the New York State Heavy Metals Registry compared to a reference group of bus drivers (Lin *et al.* 1998).

A number of studies of fetal growth include measures of exposure other than blood Pb data. Data from several studies suggest that maternal bone Pb may be more consistently associated with reduced fetal growth compared to blood Pb; however, the data are restricted to a single population. In a series of studies of women from one of three hospitals in Mexico City, higher maternal bone Pb measurements, but not maternal blood Pb levels, were associated with lower measures of fetal growth (Gonzalez-Cossio *et al.* 1997, Hernandez-Avila *et al.* 2002, Kordas *et al.* 2009, Cantonwine *et al.* 2010b). Higher maternal tibia Pb (mean, 9.8 µg/g) was associated with lower birth weight at levels >15.1 µg/g (Gonzalez-Cossio *et al.* 1997) and shorter birth length at levels >16.6 µg/g (Hernandez-Avila *et al.* 2002). Higher maternal patellar Pb (mean, 14 µg/dL) was associated with smaller head circumference at levels >24.7 µg/g (Hernandez-Avila *et al.* 2002). In further study the authors reported that the H62D genotype may enhance the adverse effect of Pb (Cantonwine *et al.* 2010b), and folate may decrease the adverse effect of Pb (Kordas *et al.* 2009). In a study of 100 mother-infant pairs in France, maternal and infant hair Pb levels were not associated with small for gestational age (Huel *et al.* 1981).

Several studies have investigated the association between placental Pb levels and fetal growth. Two studies (n=53 and n=79, respectively) reported a relationship between higher placental Pb and lower birth weight, shorter length, and/or smaller head circumference (Ward *et al.* 1990, Gundacker *et al.* 2010). Two case-control studies reported higher placental Pb levels in births with fetal growth restriction (n=20) (Llanos and Ronco 2009) or intrauterine growth restriction (referred to as intrauterine growth retardation by the authors) (n=50) (Richter *et al.* 1999). Three studies reported a lack of an association between placental Pb and birth weight: 161 women from the Yugoslavia Prospective Study (Loiacono *et al.* 1992); 262 women constituting a combined population from Russia and Norway (Odland *et al.* 2004); and 126 births at the Birmingham Maternal Hospital (Wibberley *et al.* 1977). It is difficult to determine of usefulness of placental Pb as a measure of exposure rather than blood Pb for low birth weight because most studies do not report exposure data for both measures, and the results are inconsistent. In the one study that did include both placental Pb and maternal blood Pb levels (Gundacker *et al.* 2010), low birth weight was associated with both maternal blood Pb levels and placental Pb, as described above.

Summary of support for conclusions

Animal data support an association between Pb and lower birth weight at high blood Pb levels (54-300µg/dL in squirrel monkeys, mice, and rats; see U.S. EPA 2006 for recent reviews of the animal data, ATSDR 2007). As described above, a number of epidemiological studies report effects of Pb on fetal growth or birth weight. The conclusion of *sufficient* evidence that maternal blood Pb <10 µg/dL is associated with reduced fetal growth is based on four prospective studies with maternal blood Pb during pregnancy, a large retrospective cohort study, and a number of cross-sectional studies with maternal or umbilical cord blood Pb at delivery. Although the results are not entirely consistent across studies, and some studies

report that prenatal blood Pb levels <10 µg/dL are not associated, the supporting studies provide sufficient evidence that maternal blood Pb levels <10 µg/dL are associated with reduced fetal growth. In particular, the large retrospective study of 43,288 mother-infant pairs from the New York State Heavy Metals Registry contributes substantially to the conclusion of *sufficient* evidence because it is based on such a large cohort with a low mean blood Pb level (2.1 µg/dL), and the analyses adjust for multiple potential confounders, including maternal age, race, parity, smoking, drug abuse, infant sex, and participation in financial assistance as a measure of socioeconomic status (Zhu *et al.* 2010). Additional support is provided by a number of cross-sectional studies with maternal or umbilical cord blood Pb <10 µg/dL at delivery, as well as a group of studies from a single group that demonstrate a relationship between higher maternal bone Pb and lower fetal growth. The conclusion of *inadequate* evidence that paternal blood Pb at any level is associated with fetal growth is based on a small number of studies and general lack of observed effect. The NTP conclusion of *sufficient* evidence for effects of maternal blood Pb <10 µg/dL on fetal growth is stronger than the 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) and the 2006 EPA AQCD for Lead (U.S. EPA 2006), in which the evidence was characterized as inconsistent. The NTP conclusion of *inadequate* evidence for effects of parental blood Pb on fetal growth is consistent with the 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) and the 2006 EPA AQCD for Lead (U.S. EPA 2006).

8.3.8 Preterm Birth and Gestational age

There is *limited* evidence that maternal blood Pb levels <10 µg/dL are associated with preterm birth or reduced gestational age, because of inconsistent results in studies with low blood Pb levels. Increasing maternal blood Pb levels during pregnancy were associated with preterm birth or reduced gestational age in two prospective studies (Cantonwine *et al.* 2010a, Vigeh *et al.* 2011) and five cross-sectional studies (Satin *et al.* 1991, Fagher *et al.* 1993, Odland *et al.* 1999, Torres-Sanchez *et al.* 1999, Patel and Prabhu 2009) with exposure data from maternal or umbilical cord blood Pb at delivery with mean blood Pb levels <10 µg/dL (see the Preterm section of Appendix E: Reproductive and Developmental Effects). However, a number of cross-sectional studies and several prospective studies (e.g., Bornschein *et al.* 1989, Sowers *et al.* 2002) report no association between maternal blood Pb and preterm birth at the same blood Pb levels. In addition, the large retrospective study of 43,288 mother-infant pairs from the New York State Heavy Metals Registry did not find an association between maternal blood Pb (mean, 2.1 µg/dL) and preterm birth (Zhu *et al.* 2010). There is *inadequate* evidence that paternal blood Pb at any level is associated with preterm birth, because of the small number of studies and general lack of observed effects in studies that report blood Pb levels in men.

Studies that compare maternal blood Pb levels during pregnancy with preterm births report mixed results; four studies found an association between blood Pb (mean, 4-10 µg/dL) and preterm birth or decreased gestational age, and three did not find an effect of blood Pb (mean, 1-23 µg/dL). The data are also inconsistent when examined by maternal Pb levels from a specific trimester. Higher maternal blood levels (mean, 6-7 µg/dL) in the first and second trimester, but not the third trimester, were associated with decreases in gestational age in a prospective study of 327 women in Mexico City (Cantonwine *et al.* 2010a). Higher maternal blood levels in the first trimester was associated with preterm birth and with decreases in

gestational age in a prospective study of 348 women with mean blood of 4 µg/dL in Iran (Vigeh *et al.* 2011). Maternal blood Pb ≥10 µg/dL was associated with preterm birth in a study of women in the California Pb surveillance program (n=262); however, in contrast to the data from Mexico City and Iran, the effect was significant during the second and third trimesters, but not during the first trimester (Jelliffe-Pawlowski *et al.* 2006). Higher maternal blood Pb levels (mean, 8.3 µg/dL) sampled at the first prenatal visit in women (n=185) from the Cincinnati Lead Study were correlated with decreases in gestational age (Dietrich *et al.* 1987); the results were reported as part of an analysis of neurological data, and the study group was a subset of women evaluated in a later study that did not find a significant association (Bornschein *et al.* 1989). Maternal blood Pb levels (mean, 7.5 µg/dL) at 16-28 weeks (second trimester into start of third trimester) of gestation were not associated with gestational age (Bornschein *et al.* 1989). Maternal blood Pb (mean, 1.1 µg/dL) sampled at 12 weeks (first trimester), 20 weeks (second trimester), and 28 weeks (third trimester) of gestation in 705 women in Camden, NJ, was not associated with preterm birth (Sowers *et al.* 2002). Maternal blood Pb levels at mid-pregnancy (second trimester, mean=20 µg/dL) and delivery (mean, 23 µg/dL) were not associated with preterm birth in women from the Yugoslavia Prospective Study (n=907) (Factor-Litvak *et al.* 1991). Excluding the Dietrich *et al.* (1987) study, three prospective or retrospective studies support an association between maternal blood Pb from 4 to 10 µg/dL during pregnancy and preterm birth (Jelliffe-Pawlowski *et al.* 2006, Cantonwine *et al.* 2010a, Vigeh *et al.* 2011), and three studies do not support an association with maternal blood Pb (Bornschein *et al.* 1989, Factor-Litvak *et al.* 1991, Sowers *et al.* 2002). The studies that do not support a relationship with Pb exposure are older, but all of these studies considered and adjusted for potential confounders, including maternal age, smoking, and other factors. The studies that do not support a relationship with Pb exposure also have larger sample sizes (n= 705-907) than the studies that support an effect of Pb (n=262-348).

Additional studies that sampled maternal or umbilical cord blood Pb at delivery also reported inconsistent results for these indicators of Pb exposure and preterm birth. Mean Pb levels from 1 to 15 µg/dL in maternal blood or umbilical cord blood at delivery were associated with preterm birth or reduced gestational age in mother-infant pairs from studies in several locations: five cities in California (n=723) (Satin *et al.* 1991); the Port Pirie, Australia, birth cohort study (n=721) (McMichael *et al.* 1986); Rolla and Columbia, MO (n=502) (Fahim *et al.* 1976); Mexico City (n=620) (Torres-Sanchez *et al.* 1999); a combined population from Russia and Norway (n=262) (Odland *et al.* 1999); Glasgow (n=236) (Moore *et al.* 1982); a hospital in India (n=205) (Patel and Prabhu 2009); and a small combined population from Poland and Sweden (gestational age) (n=17 preterm and n=13 controls)(Fagher *et al.* 1993). Mean Pb levels from 2 to 30 µg/dL in maternal blood at delivery or umbilical cord blood were not associated with preterm delivery or reduced gestational age in mother-infant pairs from the New York State Heavy Metals Registry (n=43,288) (Zhu *et al.* 2010); the Brigham and Women's Hospital (n=3,503) (Bellinger *et al.* 1991); Louisville, KY, General Hospital (n=635) (Angell and Lavery 1982); Memphis (n=102) (Jones *et al.* 2010); or New York City (n=100) (Rajegowda *et al.* 1972). The database of studies that determined exposure from maternal or umbilical cord blood Pb at delivery includes a number of studies that do not report appropriate adjustments (e.g., Fahim *et al.* 1976), but there are also positive (e.g., Torres-Sanchez *et al.* 1999) and negative studies

(e.g., Zhu *et al.* 2010) for the effects of Pb that adjusted for potential confounders, including maternal age, parity, and smoking.

Few studies address the relationship between paternal blood Pb and preterm births, and the available data do not support a relationship between blood Pb in men and preterm birth. In a study of over 3,000 births to male workers in the New York State Heavy Metals Registry, Lin *et al.* (1998) reported that parental blood Pb >25 µg/dL did not affect the relative risk of preterm births (RR=0.89 (95% CI: 0.64, 1.26)) compared to a reference group of bus drivers; however, continued blood Pb >25 µg/dL for more than 5 years was associated with increased relative risk of preterm births (RR=3.03 (95% CI: 1.35, 6.77)) compared to workers that did not consistently report a blood Pb level >25 µg/dL. In a similar worker surveillance program in China, paternal blood Pb (mean, 14 µg/dL) was not associated with preterm birth (Chen *et al.* 2006). Paternal Pb exposure determined by paternal job category was also not associated with preterm birth in studies of members of the printers' unions in Oslo, Norway (n=6,251 births) (Kristensen *et al.* 1993) or births from the National Natality Survey and Fetal Mortality Survey in the United States (Savitz *et al.* 1989). Occupational exposure in Norway with possible paternal Pb exposure (n=35,930 births) was associated with longer-term births, and maternal Pb exposure was associated with preterm birth (Irgens *et al.* 1998).

Several studies also address other measures of exposure such as hair or placental Pb levels. Huel *et al.* (1981) reported higher hair Pb levels in mothers and offspring from preterm births than from normal births. Two studies reported higher placental Pb levels in preterm birth (or combined analysis of preterm births and births with premature rupture of membranes) than in births with normal delivery (Ward *et al.* 1990, Falcón *et al.* 2003). However, four other cross-sectional studies did not find a significant relationship between placental tissue Pb concentrations and preterm births (Fahim *et al.* 1976, Ward *et al.* 1987, Baghurst *et al.* 1991, Loiacono *et al.* 1992). Cantonwine (2010a) did not find a significant association between maternal plasma Pb and preterm birth, although the relationship was significant with maternal blood Pb levels.

Summary of support for conclusions

Animal data were not located that support an effect of Pb on preterm delivery, although potentially related endpoints such as pup survival and birth weight were adversely affected at high blood Pb levels (54-300µg/dL in squirrel monkeys, mice, and rats; see U.S. EPA 2006 for recent reviews of the animal data, ATSDR 2007). As described above, the human data are not consistent for an effect of Pb on preterm birth. Although a number of prospective studies with maternal blood Pb levels during pregnancy and cross-sectional studies with umbilical cord blood Pb levels at delivery reported an association between prenatal blood Pb levels <10 µg/dL and preterm birth, the conclusion of *limited* evidence is based on the inconsistent results and because a large retrospective study did not find an association between maternal blood Pb levels and preterm birth. In particular, the large retrospective study of the New York State Heavy Metals Registry included 43,288 mother-infant pairs and did not find an association between maternal blood Pb (mean, 2.1 µg/dL) and preterm birth (Zhu *et al.* 2010). The conclusion of *inadequate* evidence that paternal blood Pb at any level is associated with

preterm birth or reduced gestational age is based on a small number of studies and general lack of observed effect. Of the five studies located that address paternal exposure and preterm birth, three report no effect, one reports a Pb-associated increase in gestational age, and one reports an association with persistently elevated paternal Pb (>25 μg/dL for 5 years) and preterm birth. The NTP conclusion of *limited* evidence for effects of maternal or umbilical cord blood Pb <10 μg/dL on preterm birth is in line with the 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) and the 2006 EPA AQCD for Lead (U.S. EPA 2006), in which the evidence was characterized as inconsistent.

8.3.9 Endocrine Effects

There is *inadequate* evidence to evaluate the potential association between blood Pb and major endocrine or changes in hormone levels, because of inconsistency of effects across available studies (see Endocrine section of Appendix E: Reproductive and Developmental Effects). The data are inconsistent for the effects of Pb on luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone (T), and other hormones, including estradiol-17β (E_2), prolactin (PRL), thyroid-stimulating hormone (TSH), thyroxin (T_4), triiodothryonine (T_3), parathyroid hormone, inhibin B, and insulin-like growth factor 1 (IGF-1). There are inadequate studies of endocrine effects in general to determine if the lack of consistency in the data reflects variation in the potential interaction with Pb or whether it is due to well-known hormonal variation and cyclicality on a daily, monthly, and seasonal basis.

In studies that examined the relationship between blood Pb and LH or FSH, the results are inconsistent. Blood Pb levels (mean, 30-69 μg/dL) were not associated with serum levels of LH or FSH in a number of studies of men with occupational Pb exposure (Assennato *et al.* 1986, Ng *et al.* 1991, Gennart *et al.* 1992a, Alexander *et al.* 1996b, Telisman *et al.* 2000, Mahmoud *et al.* 2005, Naha and Manna 2007, Hsieh *et al.* 2009) and not at lower blood Pb levels (mean, 3-10 μg/dL) in men or women recruited at infertility clinics (Chang *et al.* 2006, Meeker *et al.* 2010, Mendiola *et al.* 2011) or men without occupational Pb exposure (Telisman *et al.* 2007). Basal levels of LH and FSH did not differ between Pb workers and a reference group; however, gonadotropin-releasing hormone-stimulated FSH was decreased in Pb workers with median blood Pb levels of 31 μg/dL (Erfurth *et al.* 2001). In a study of 23 Pb workers serum (mean, 60-73 μg/dL), LH was increased compared to a reference group (mean, 17 μg/dL) (Rodamilans *et al.* 1988). Several studies have reported that FSH was increased (Cullen *et al.* 1984, McGregor and Mason 1990, McGregor and Mason 1991, De Rosa *et al.* 2003) or decreased (Gustafson *et al.* 1989) in men at blood Pb levels of 20-45 μg/dL compared to a reference group. Results of a study by Krieg *et al.* (2007) of women from NHANES III suggest that the effect of Pb on FSH and LH is modified by reproductive status (e.g., menopausal status) or external hormones. FSH was increased with increasing blood Pb in women from NHANES III (mean blood Pb, 2.8 μg/dL) but decreased with increasing blood Pb in women taking birth control pills (Krieg 2007). LH was increased with increasing blood Pb in postmenopausal women from NHANES III, and LH was not associated with blood Pb in other women (Krieg 2007).

The data are inconsistent for the effects of Pb on T, thyroid hormones (TSH, T_4, and T_3), and for other hormones, including E_2 and PRL. Blood Pb levels (mean, 30-69 μg/dL) were not associated

with serum T levels in a number of studies of men with occupational Pb exposure (Assennato *et al.* 1986, Ng *et al.* 1991, Gennart *et al.* 1992a, Alexander *et al.* 1996b, Mahmoud *et al.* 2005, Naha and Manna 2007, Hsieh *et al.* 2009). Several studies have reported lower basal or stimulated T with occupational exposure in the same blood Pb range (Braunstein *et al.* 1978, Rodamilans *et al.* 1988, Telisman *et al.* 2000). At lower blood Pb levels (<10 µg/dL), exposure was associated with increased serum T in a study of 240 men without occupational Pb exposure (Telisman *et al.* 2007) and in 219 men recruited from infertility clinics, but not after adjustment for exposure to other metals (Meeker *et al.* 2010). However, Mendiola *et al.* (2011) did not find an association between blood Pb (mean, 10 µg/dL) and serum T in 60 men recruited at an infertility clinic. Occupational Pb exposure in men at mean blood Pb levels 30-40 µg/dL were not associated with E_2 in one study (Mahmoud *et al.* 2005) but was associated with decreased E_2 in another study (Telisman *et al.* 2000). At blood Pb levels <10 µg/dL, Pb was associated with increased E_2 in a case-control study of women recruited at an infertility clinic (n=64) and controls from a postpartum clinic (n=83) in Taiwan (Chang *et al.* 2006). Also, at blood Pb levels <10 µg/dL, E_2 was increased and PRL was decreased in association with Pb in men without occupational Pb exposure (Telisman *et al.* 2007). In men with occupational exposure to Pb and mean Pb levels from 30 to 60 µg/dL, blood Pb was not associated with PRL or did not differ from a reference group (Assennato *et al.* 1986, Roses *et al.* 1989, Ng *et al.* 1991, Telisman *et al.* 2000). Serum TSH was elevated in Pb gas station workers with blood Pb mean of 51 µg/dL compared to a reference group (Singh *et al.* 2000). In contrast, higher blood Pb levels were related to lower TSH in women, but not in men, among people in Quebec that regularly eat freshwater fish; T_3 and T_4 were not associated with Pb levels <10 µg/dL (Abdelouahab *et al.* 2008). Serum levels of TSH, T_3, or T_4 did not differ between men with high occupational Pb (mean, 51 µg/dL) and a reference group (21 µg/dL) (Gennart *et al.* 1992a); in male Pb workers with mean blood Pb of 31 µg/dL (Erfurth *et al.* 2001); in male Pb workers with mean blood Pb of 24 µg/dL (Schumacher *et al.* 1998); in a study of 24 newborns with mean blood Pb levels of 6 µg/dL (Iijima *et al.* 2007); or in 68 children <8 years of age with range of blood Pb from 2 to 77 µg/dL (Siegel *et al.* 1989). Two studies of men with occupational Pb exposure (mean Pb level, 42-51 µg/dL) reported opposite results: T_4 and free T_4 were higher in 75 Pb workers than in 68 matched controls (Lopez *et al.* 2000); lower T_4 and free T_4 were associated with higher blood Pb in a study of 54 workers at a brass foundry (Robins *et al.* 1983). In 309 mother-children pairs from the Yugoslavia Prospective Study, higher maternal T_4 was associated with lower blood Pb in women from a Pb-smelting town (median blood Pb, 20 µg/dL) but not in a reference town (median blood Pb, 6 µg/dL) (Lamb *et al.* 2008). Cumulative Pb exposure was associated with increased serum inhibin B levels in two studies of male Pb workers (n=181 (Hsieh *et al.* 2009); n=68 (Mahmoud *et al.* 2005)).

There are few studies of Pb and hormone levels in children. Two available studies suggest that Pb may decrease LH and FSH. In a study of 41 children 10-13 years of age in Egypt, boys and girls with blood Pb >10 µg/dL had lower FSH and LH; boys had lower serum T, but there was no effect of Pb on E_2 (Tomoum *et al.* 2010). Similarly, Vivoli *et al.* (1993) reported decreased LH and FSH in boys with blood Pb ≥10 µg/dL in a study of 418 children in Italy; T and E_2 were not related to blood Pb levels. Girls 6-11 years of age in NHANES III with blood Pb ≥1 µg/dL had lower levels of inhibin B (Gollenberg *et al.* 2010).

Summary of support for conclusions

Animal data support an association between Pb exposure and altered hormones, particularly decreased LH, FSH, and E_2 in females, at high blood Pb levels (30-300µg/dL in Cynomolgus monkeys and rats; see U.S. EPA 2006 for recent reviews of the animal data, ATSDR 2007). Rodent data also support an effect of Pb testosterone in some studies; however, even within the animal literature, increase, decrease, and lack of effect are all reported. Animal data also support decreased IGF-1, LH, and E_2 as potential mechanisms for the Pb-associated delay in puberty. Animal data also support an effect of developmental Pb exposure on the hypothalamic-pituitary-adrenal axis and changes in the stress response or basal corticosteroids. The human data on potential associations between Pb and LH, FSH, and T are inconsistent, and for other hormones the data are inconsistent as well as limited in nature. Therefore, the NTP determined there was *inadequate* evidence to conclude that blood Pb was associated with specific hormone changes. The conclusion of *inadequate* evidence for endocrine effects of Pb in humans is in line with the discussion of the general inconsistencies in the human database with the ATSDR and EPA; however, the 2006 EPA AQCD for Lead (U.S. EPA 2006) states that the toxicological data (animal data) support Pb as an endocrine disruptor in males and females at various points along the hypothalamic-pituitary-gonadal axis. Additional data, particularly data from prospective studies with appropriate consideration and control for timing in the reproductive cycle, age, and other factors, are required to clarify the potential relationship between blood Pb and endocrine parameters.

8.3.10 Congenital Malformations

There is *inadequate* evidence to evaluate the potential association between blood Pb and congenital malformations. Few studies investigate the potential association between Pb exposure and malformations, and even fewer have blood Pb or other biological monitoring data (see the Congenital Malformations section of Appendix E: Reproductive and Developmental Effects). Of the studies with blood Pb data, only one supports an association between blood Pb levels and congenital malformations: in a retrospective analysis, Needleman *et al.* (1984) found a higher relative risk of minor congenital anomaly with higher all umbilical cord blood Pb levels (6.3 µg/dL: RR=1.87 9 (1.4, 2.4); 15 µg/dL: RR=2.39 (1.7,3.4); 24 µg/dL: RR=2.73 (1.8, 4.2)). There are few other published studies; most lack blood Pb data or other biomarkers, and exposure is based on job categories or indirect measures. For example, residence in the ceramic district in Italy, an area known for higher Pb exposure levels, was associated with increased relative risk of congenital malformations (RR=1.48 (95% CI: 1.15, 1.89)), including hydrocephalus, oral clefts, cleft lip, and malformations of the ear, heart, cardiovascular system, musculoskeletal system, and integument (Vinceti *et al.* 2001).

Several occupational studies investigated the relationship between paternal blood Pb and congenital malformations, and the available evidence is inconsistent. Blood Pb levels determined from monitoring data for 929 employees of the Cominco smelter in British Columbia were not associated with odds ratio for still births and birth defects combined (Alexander *et al.* 1996a). Paternal occupational Pb exposure was not associated with congenital malformations in a study of 764 workers at a copper smelter that compared rates of

malformations between pregnancies after employment to pregnancies that took place before occupational Pb exposure (Beckman and Nordstrom 1982). Estimated paternal blood Pb levels based on job categories were associated with an increased odds ratio for congenital malformations (OR=3.2 (95% CI: 1.0, 10.2)) when evaluated along with paternal smoking (Sallmen *et al.* 1992).

A number of studies have examined the potential association between Pb exposure and neural tube defects. The evidence that Pb exposure is associated with neural tube defects is inconsistent, and both studies that include blood Pb data do not support an association with Pb exposure: umbilical cord blood Pb at birth in a case-control study of mother-infant pairs in Turkey (Zeyrek *et al.* 2009), and maternal blood Pb taken 5-6 weeks after birth in Mexican-Americans living near the Texas-Mexico border (Brender *et al.* 2002, 2006). Dawson *et al.* (1999) reported higher Pb levels in amniotic fluid of neural tube defect cases (n=11) than in controls (n=29). In a study that looked for serious birth defects among births in Norway with possible parental occupational Pb exposure by job classification, Irgens *et al.* (1998) reported that maternal Pb exposure (of n=1,803 exposed births) was associated with a greater odds ratio for neural tube defects (OR=2.87 (95% CI: 1.05, 6.38)) but not paternal exposure (n=35,930 exposed births). Bound *et al.* (1997) reported a significant association between risk of neural tube defects in a case-control study that determined Pb exposure by residence in a district in the United Kingdom known to have higher drinking water levels of Pb. Drinking water levels of Pb or residence near a hazardous waste site with known Pb were not associated with neural tube defects or anencephalus in several case-control studies (Elwood and Coldman 1981, Croen *et al.* 1997, Macdonell *et al.* 2000).

Several studies have examined the potential association between Pb exposure and cardiovascular defects. The evidence that Pb exposure is associated with cardiovascular defects is inconsistent, comes from few studies, and lacks good exposure data. The study of residents in the Pb-associated ceramic district in Italy described above reported increased relative risk of prevalence of specific heart malformations (RR=2.47 (95% CI: 1.57, 3.70)) and general cardiovascular malformations (RR=2.59 (95% CI: 1.68, 3.82)) (Vinceti *et al.* 2001). Drinking water levels of Pb and residence near a hazardous waste site with known Pb were not associated with congenital heart disease or cardiovascular anomalies in several case-control studies (Zierler *et al.* 1988, Aschengrau *et al.* 1993, Croen *et al.* 1997). A case-control study of 54 children with total anomalous pulmonary venous return (TAPVR) and 522 matched controls in the Baltimore-Washington Infant Study reported significant odds ratio for paternal Pb exposure (OR=1.83 (95% CI: 1.00, 3.42) (Jackson *et al.* 2004)).

Several studies have also examined the potential association between Pb exposure and cleft lip or cleft palate. The evidence that Pb exposure is associated with oral clefts comes from few studies, all of which lack biological exposure data. The study of residents in the Pb-associated ceramic district in Italy described above reported increased relative risk of oral clefts (RR=2.28 (95% CI: 1.16, 4.07)) and cleft lip (RR=2.43 (95% CI: 1.13, 4.62)) (Vinceti *et al.* 2001). A case-control study of 100 mothers of babies with oral clefts and 751 controls reported increased odds ratio of oral clefts (OR=4.0 (95% CI: 1.3, 12.2)) (Lorente *et al.* 2000). Paternal Pb

exposure by job category among 6,251 births to male members of printers' unions in Oslo, Norway, was associated with an increased standardized morbidity ratio for cleft lip in boys (SMR=4.1 (95% CI: 1.8, 8.1)) (Kristensen *et al.* 1993).

Summary of support for conclusions

Limited animal data support an effect of Pb on congenital malformations, mainly tail defects and general external malformations in NOS rats, although increased fetal mortality was reported in some studies at high blood Pb levels (54-300µg/dL in squirrel monkeys and mice; seeU.S. EPA 2006 for recent reviews of the animal data, ATSDR 2007). In the human data, there are few studies of congenital malformations with parental Pb exposure data. Although studies have reported general effects on congenital malformations, and specific effects on neural tube defects, cardiovascular defects, and oral clefts, the results are inconsistent and the studies generally lack biological exposure data. The NTP determination that there is *inadequate* evidence to conclude parental blood Pb levels are associated with congenital malformations is consistent with the 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) and the 2006 EPA AQCD for Lead (U.S. EPA 2006).

8.4 Conclusions

The NTP concludes there is *sufficient* evidence that blood Pb levels <10 µg/dL are associated with adverse effects on development in children and that blood Pb levels <5 µg/dL are associated with adverse effects on reproduction in adult women (see **Table 8.6** for complete list of reproductive and developmental effects conclusions). In some studies blood Pb levels ≤2 µg/dL are associated with adverse effects (e.g., Wu *et al.* 2003, Denham *et al.* 2005 for delayed onset of puberty), and the ability to discriminate effects at the lower dose may depend on the availability of a reference group with lower blood Pb levels or the precision of blood Pb measurements. In children, there is *sufficient* evidence that blood Pb levels <10 µg/dL are associated with delayed puberty and decreased postnatal growth and *limited* evidence that delayed puberty is associated with blood Pb levels <5 µg/dL. In adults, there is *sufficient* evidence that maternal blood Pb levels <5 µg/dL are associated with reduced fetal growth and *limited* evidence that maternal blood Pb levels <10 µg/dL are associated with spontaneous abortion and preterm birth. In men there is *sufficient* evidence that blood Pb levels ≥15 µg/dL are associated with adverse effects on sperm or semen and that blood Pb levels ≥20 are associated with delayed conception time. There is *limited* evidence that blood Pb levels ≥10 µg/dL in men are associated with other measures of reduced fertility and that blood Pb levels >31 µg/dL are associated with spontaneous abortion.

Table 8.6: NTP conclusions on reproductive and developmental effects of low-level Pb				
Health Effect	**Population or Exposure Window**	**NTP Conclusion**	**Blood Pb Evidence**	**Bone Pb Evidence**
Delayed puberty	Prenatal	*Inadequate*	No data	No data
	Children	*Sufficient*	Yes, <10 µg/dL	No data
		Limited	Yes, <5 µg/dL	
Postnatal growth	Prenatal	*Limited*	Yes, <10 µg/dL	One study
	Children	*Sufficient*	Yes, <10 µg/dL	One study available, no evidence of an association
Sperm parameters	Children	*Inadequate*	No data	No data
	Men	*Sufficient*	Yes, ≥15 µg/dL	No data
Fertility / delayed conception time	Men: time to conception	*Sufficient*	Yes, ≥20 µg/dL	No data
	Men: fertility	*Limited*	Yes, ≥10 µg/dL (one study)	No data
	Women	*Inadequate*	Unclear	No data
Spontaneous abortion	Men	*Limited*	Yes, >31 µg/dL	No data
	Women	*Limited*	Yes, <10 µg/dL	No data
Stillbirth	Adults	*Inadequate*	Unclear	No data
Reduced fetal growth and lower birth weight	Men	*Inadequate*	Unclear	No data
	Women	*Sufficient*	Yes, <5 µg/dL	Yes, tibia
Preterm birth and gestational age	Men	*Inadequate*	Unclear	No data
	Women	*Limited*	Yes, <10 µg/dL	No data
Endocrine effects	Adults	*Inadequate*	Unclear	One study
Birth defects	Adults	*Inadequate*	Unclear	No data

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